Project No.
Book No.
TITLE SDS gel on PDMB Heparin

From Page No.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

mW 15λ 200

I' DH10B/RK2 P190

I' TF1 P181 200

Am SO4 resus 362 µ/l 1.5
5200 50 µ/l 105
Blue 62
Heparin frns
105 150
106 150
107 150
108 150
109 150
110 150
111 150
112 150
113 150
114 150
115 150

#4,5 sat
No TCA ppt

TF1 10+#TF3 10 10A -502 150
µ/l
H2O 100 100 - - 238 150

Vf = 300

15% TCA 300 → - - 300
(P72,9 and P50,7)
30 µl

1x sample buffer 3550 µl 405 → 35

To Page N

Witnessed & Understood by me, Deeana Polys
Date 5/1/95
Invented by
Record d by
Dat 4-28-95

PAGE 5 OF NOTEBOOK WAS BLANK

Exhibit **81**

Project No Appl. No. 09/558,421

Book No._______ **TITLE** Storage buffer

From Pag No.__

buffer F, G as per rTaq 91342.PRP

made F, 4L first as follows.

make vol up to ~~2700~~ 3200 ml (i.e. 80% of Vf of

remove 160 ml and add

20 ml Tween 20 (Pierce)

20 NP40 (Pierce)

ml 200

= buffer G

Take the remaining buffer up to Vf = 3800

for 1X buffer F

---

pool frn 105-114 of Heparin (P.1-4)

= 90 ml vol (actually measured 87 ml)

Dialyze against 2 buffer F, 5 hr

2 ml C O/N

4-30

Recovered 33 ml after Dialysis.

combine with 33 ml buffer G = Vf 66.

labeled: Tf1 DNA pol in storage buffer 4-30-95 store at -20

4.33 u/μl exp 8

To Pag N

Witn ssed & Understood by me, Deanna Rohrs Date 5/1/95

Inv nted by [signature] Date 4-29-95 4-30-95

R c rd d by

From Page No.

Buffer F, G as per rTaq 9134Z.PRP

make F, 4L first as follows.

make Vol up to ~~3700~~ 3200 ml (i.e. 80% of Vf of

remove 160 ml and add
20 ml Tween 20 (Pierce)
20 NP40 (Pierce)
ml 200
= buffer G

Take this remaining buffer up to Vf = 3700
for 1X buffer F

---

pool frm 105-114 of Heparin (P.1-4)
= 90 ml vol (actually measured 87 ml)
Dialyze against 2 buffer F, 5 hr
2 ml C O/N

4-30

Recovered 33 ml after Dialysis-
combine with 33 ml buffer G = Vf 66
labeled: TF1 DNA pol in storage buffer 4-30-95
store at -20

4.33 u/l
pol B

T Pag N

Witnessed & Understood by me,	Date	Invented by	Dat
Deanna Romp	5/1/95	Recorded by	4-29-95 / 4-30-95

Unit assays for Tfi I

Exhibit 83
Appl. No. 09/558,421

Pr j ct N .________
Book No.________

ge N .__	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	27
storage buffer (-30.9)																												
tion*																												
50	2				2				2				2															
00		2				2				2				2														
000			2				2				2				2													
000				2				2				2				2												
t 30809B (see P181)																												
50																	2											
00																		2										
00																			2									
00																				2								
310/0A-502 4-25-95 P172,9 SDS gel																												
50																					2							
00																						2						
00																							2					
400																								2				
4-28-95 SDS gel																												
50																									2			
00																										2		
00																											2	
00																												2
10x rxn mix (0,9)	48 µl →																48 →											
unit assay mix													48 →															

74°C, 10', → 10 µl 0.5 M EDTA → spot 40 µl on GFC

Tubes # 13-16 use same serial dilution as tubes # 1-4
all 3 independent serial dilutions in Taq dil buffer

To Page No.______

ed & Understood by me,	Date	Invented by	Date
[signature]	5/1/95	[signature]	5-2-95
		Recorded by	

Project No.______
Book No.______ TITLE ______

From Page No.__

	SAM	CPM1	pmol-200 BKGD	u/μl		
dil 1	1	4167.00	102.8	3.85		
	2	2383.00		4.24	4.25 ave	
	3	1401.00		4.67		
	4	1060.00		(6.68)	→	
	5	4115.00		3.80		
dil 2	6	2316.00		4.1		
	7	1394.00		4.6	4.38 ave →	4.33 u/λ
	8	846.00		5.0		
	9	4180.00		3.9		
	10	2416.00		4.3		
dil 3	11	1226.00		5.38	4.37 ave →	286,000
	12	888.00		5.3		66 ml
	13	4556.00		4.22		
Taq Rxn mix	14	2720.00		4.89	4.66 ave ←	ave of 500
	15	1452.00		4.86		DNA substrate
(500 ng of DNA)	16	1330.00		(5.3)		Taq Rxn mix
	17	6287.00		1.18		= 8.0
	18	4373.00		1.62	1.6 u/λ ave	unit
30709B	19	2428.00		1.73		
	20	1302.00		1.87		= 30
	21	13554.00		2.59		total
1010A	22	8692.00		3.5	3.5 u/λ	units
	23	5151.00		3.8		
	24	3002.00		4.36		
	25	12756.00		2.44		
1010A	26	8623.00		3.27	3.51 u/λ	
	27	5397.00		4.04		
	28	2964.00		4.30		
BKGD = 200	29	194.00				
	30	96542.00	57.9 cpm/pmol			

5-2-95 label Heparin frn of 4-30-95 at

4.33 units/λ

To Pag N

Witnessed & Understood by me, [signature] | Date 5/15/95 | Invented by [signature] | Recorded by | Date 5-2-95

e N . follow + Taq QC : 10342 . QCP

	22 Rxns	ss DNA Rxn mix	ds DNA Rxn mix	
CR buffer		110 µl	110 µl	✓
M Tris (4@ pH 8.4)				
mM KCl				
mM $MgCl_2$		110	110	✓
74 (+) ssDNA 0.2µg/l		110 µl		✓
174 RF 0.33 µg/l			66.7	✓
@ H_2O		666 / 990	703.3 / 990	✓

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

-30.55 .48 .92 1.39 1.8-2.3
5u/l

.57 1.14 1.7 2.3 2.9
micrin
0A-502
5/l (P8)

rude ext
I 4-13-95)
k
10,000 1
1,000 1
100 1
10 1
0 dil

4.5 4 3.6 3.15 2.7 4.X 3.9 3.3 2.7 2.1 4 4 4 4 4 5 - 5 ✓

il buffer

Vf=50µl 72°C, 3 hr (start 12:10 PM)

- 17 is 45µl ss ΦX DNA Rxn mix and #18-34 is ds DNA mix 45µl Rxn mix

To Page No.

ed & Understood by me,	Date	Inv nted by	Date
Blanco	5/15/95	R c rd d by	5-3-95

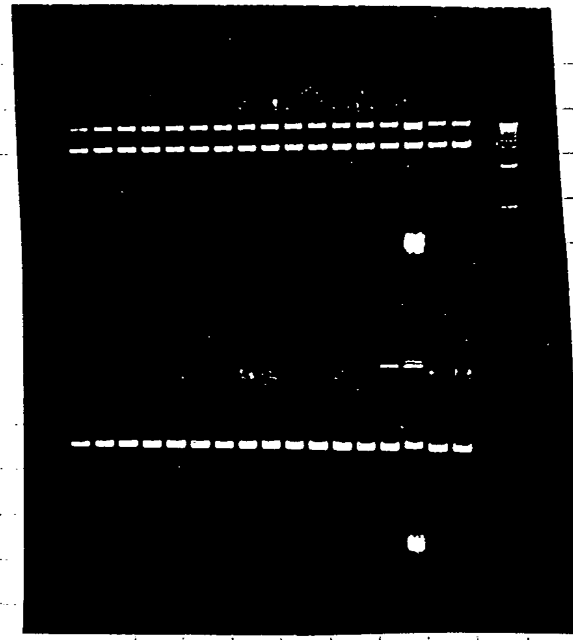
Project No.________
Book No.________ TITLE ____________________

From Page No.__

CTI Tf1 | Epicentre | crude ext FrI

units 2 4 6 8 10 | 2 4 6 8 10 | blank | dil buffer blank

(relaxed) RFII →
scDNA →



} dsDNA endo

} ssDNA endo

note for RF ϕx substrate with positive control (FrI) there is a slight increase in relaxed circles (ie nicks) and immediate conversion to small fragments. no nicking seen for either ssDNA or dsDNA substrates by any Tf1 or Epicentre.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	5/15/95	[signature] Recorded by	5-3-95

Dilutions of Taq for PCR functional assay

ge No.

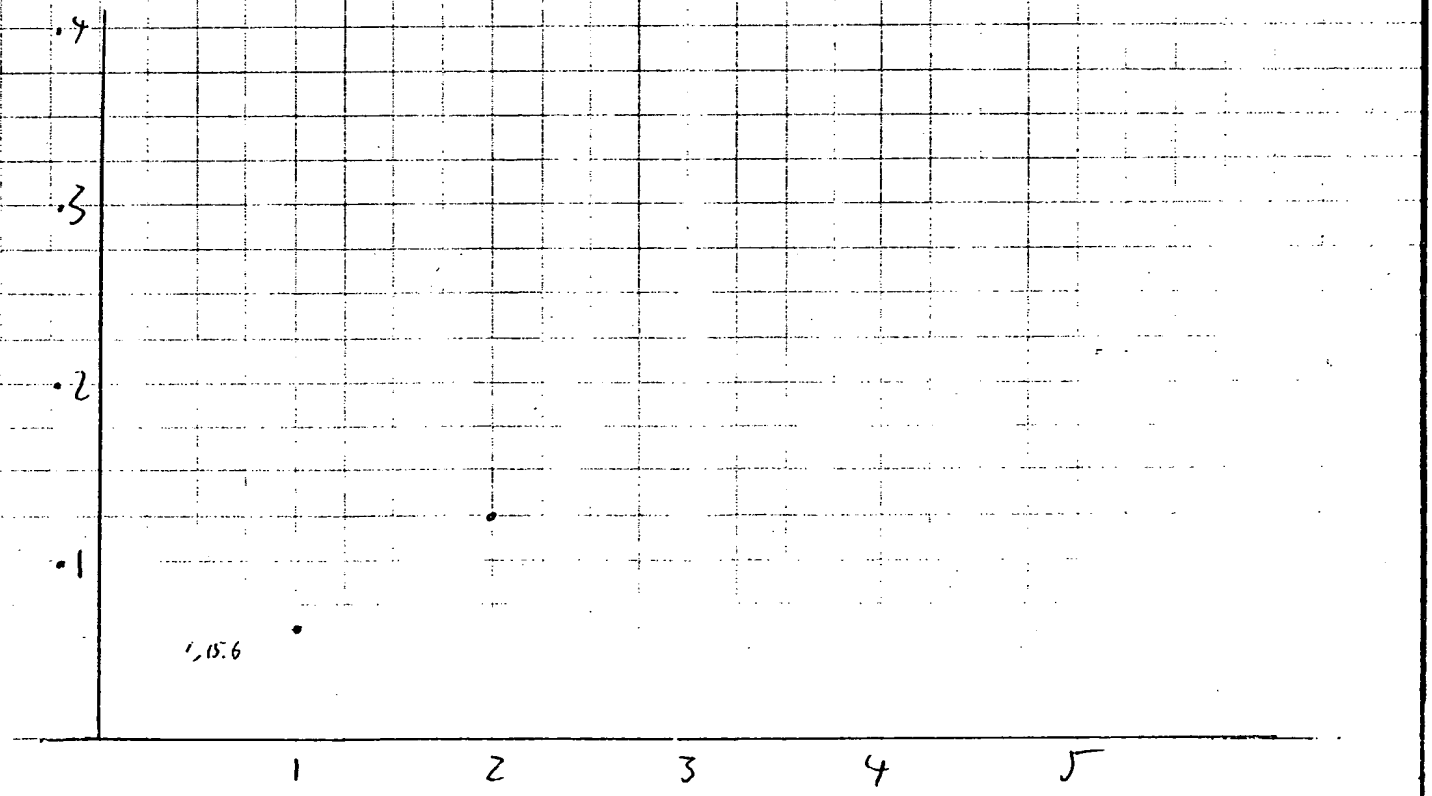
	#	1	2	3	4	5		1	2	3	4	5	
Cf u/µl		.0625	.125	.188	0.25	0.5							
Lot# EM7414		5	5	5	5	5		3	3	3	3	3	4
dilution buffer		395	195	128.3	95	45		237	117	77	57	27	36
Vf =		400	200	133.3	100	50		240	120	80	60	30	40

R401

	#	6	7	8	9	10

		11	12	13	14	15

plitaq



To Page No.

ed & Understood by me,	Date	Invented by	Date
Polano	5/15/95	R cord d by	

Exhibit 86
Appl. No. 09/558,421

12

Projec

Book No.______ TITLE 32P "33 correct" (as done P146,9 and 136,9) and at 5µM primer Cf so 3' exo reaction can be Cf = 200 nM

From Page No.__

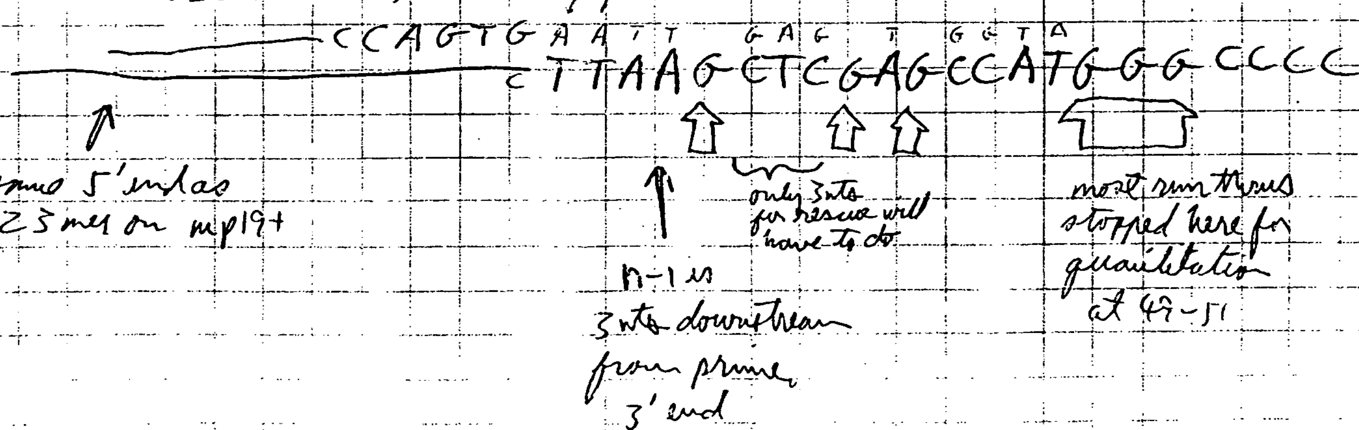
(P-137)

		H2O	10.5			
33 mer correct (P138,9)	20 pmol/λ (20µM)	7.5	15 µl	✓ ✓		~300 pmol 33mer
32P γ ATP 10 mCi/ml 5-12-95		5	30 µl	✓		11 × 10^6 cpm/µl ~90
5 x Kinase buffer		6	12 µl	✓		
PNK 10u/λ		1	3 µl	✓		
~~Hcl~~		30	60 µl	37°C, 30'		
				55°C, 5'		

Plan for fidelity assay for pol ± 3' exo

(−) dCTP (+) dA,T,G-TP

(33 correct P138,9)



To Page N

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	5/15/95	[signature]	5-9-95
		Recorded by	

... unit assay on Tne
(in Tag unit assay mix)

Project No. ______
Book No. ______

Page No.			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
5 u/l																					
.95																					
25			2																		
50				2																	
00					2																
00						2															
2000							2														
4000								2													
5-7-95																					
u/l																					
1000									2												
2000										2											
4000											2										
7000												2									
16000													2								
32000														2							
4-30-95																					
u/l																					
250															2						
500																2					
1000																	2				
2000																		2	—	—	—

	#	cpm	pmol	u	
Tne 3-2-95	1	3906.00	88.9	1.7	1.56
	2	2179.00			1.65
	3	1383.00			1.95
	4	929.00			
	5	916.00			−BKGD
	6	448.00			
Tne 5-7-95	7	8537.00	194	29.1	
	8	4263.00		29.1	33
	9	2648.00		36	
	10	1531.00		41	35
	11	776.00			
	12	570.00			
Tfl	13	4932.00	112	4.2	
	14	2336.00		4.0	
	15	1307.00			3.6
	16	1011.00			
	17	298.00	} BKGD → ave 239		
	18	266.00			
	19	153.00			
	20	105445.00	65.9 cpm/pmol		

conclude

Tne 3-2-95 ave 34 u/l
(agrees with 36 u from Liz)

Tne 3-2-95 1.72 u/l ave

To Page No. ______

Read & Understood by me,	Date	Invented by	Date
[signature]	5/15/95	[signature]	5-5-95
		Recorded by	

From Page No.__ see P136,9 137,9 for procedure: 200nM primer (
so its like a real PCR

	①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪	cocktail
10x PCR buffer 50mM MgCl2	5	→								→		60λ ✓
32P 33mer correct" pI2 (5μM) H2O	1.5μl	→								→		18λ ✓ (1.5mM
	2	→								→		24λ ✓ 102 vf - use 8.5μl/
Tfl FrI 4-13-95	10											for #⑪
Tfl FrI'/PEI 4-13-95 (2.45u/λ)		10										✓ 10μl of 5x buffer Chan
Tfl in storage buffer 4.33 u/λ (P8)			10									Min (2-14.5μl ou has dNTPs and
Tfl epicentre lot TF31010A-502 (3.5u/λ P.7)				12.4								20μl Tfl/vol 0.745λ from
rTaq 5u/λ EKBTI					10							✓ 2λ 32P 33 m
The 3-2-95 1.72u/λ (P.13) dilute to 0.5u/λ						210						✓ 18λ H2O
The 5-7-95 36u/λ (according to Liz F and see P13 where I got 34u/λ) dilute to 0.5u/λ							210					50λ vf
1% Tween20/NP40 H2O	0.5	0.5	1	2	1.5	3	1.5	3	1.5	1.5	✓	
Taq dil buffer	31	31	31	29.1	31.5	29.5	31.5	29.5	31.5	10	✓	

vf = 50x

74°C. remove 10μl to 5μl cycl seq stop
at 0.33, 1, 2 hr
run on 8% PAGE

see analyses of Tfl/vent epo rate and Tfl loss of full beg 33mer on P 46.

To Page N

Witnessed & Understood by me,	Date	Invented by	Date
	5/15/95	Recorded by	5-10-95

PAGE 15 OF NOTEBOOK WAS BLANK

Exhibit 88
Appl. No. 09/558,421

Project No. _____ ds 3'exo QC assay for Tfl
Book No. _____ TITLE 10342.QCP, 30042.SOP

From Page No. ___

	Mix A		
10 PCR buffer	110 µl	✓	Cf = 1x
50 mM $MgCl_2$	110 µl		Cf = 5 mM
3'(ds) substrate 0.5 pmol/λ lot EFE73	44 µl		(1 pmol/reacti
autoclaved, filtered H_2O	726 µl	✓	
	Vf = 990 µl		

acc to QC 103

units 0 2 4 6 8 10

tube #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
4-30-95 Tfl (P.8 4.33K/λ)	—			0.46	0.62	1.39	1.85	2.3							
Tfl epicenter lot TF31N10A502 3.5K/λ (acP 8)	—								0.5	1.4	1.1	2.2	2.2	98.2	
Tfl Fr I 4-13-95 dil none 1/100 1/10 1/10 1/100 none															
H_2O	3	9.5	4.1	3.6	3.2	2.7	5	4.4	3.9	3.3	2.7	2.1			
Tag otwasebuffer 2															✓
Mix A 45 µl →															✓
Tag dil buffer →															✓
Vf = 50 µl															

74°C, 60' 37°C, 60'

put tubes on ice

To Page N

Witnessed & Understood by me, [signature] Date 5/15/95

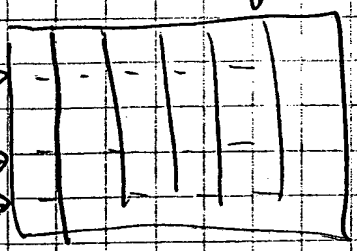
Invented by [signature] Date 5-12-95

Recorded by

cut plate in half → get 20 × 10 cm plates

use 0.9 cm width lanes
origin at 2 cm

score lanes every 0.9 cm



spot 5 µl, dry, spot another 5 µl, dry

resolve in 2 N HCl

bring solvent front to top of plate

Dry by heat lamp, not more than 7 min

count bottom = 3.2 cm (= ori)

3.2 cm – 7.6

7.6 = top

for each

∴ 3 × 18 = 54 tubes

add 3.5 ml fluor

count 3H

d & Understood by me, Dolan	Date 5/15/95	Invented by	Date 5-12-95
		Recorded by	

Project No._____

Book No._____ TITLE To released

From Page No.___

			CPM	
	1	4802.00		
0	2	673.00		
	3	92.00	BKGD	—
	4	4782.00		
2	5	709.00		
	6	123.00	31	0
Tf1	7	4935.00		
4-30-95	8	702.00		
4	9	114.00	22	0
	10	4670.00		
6	11	743.00		
	12	129.00	37	0
	13	4732.00		
8	14	669.00		
	15	123.00	31	0
	16	4788.00		
10	17	767.00		
	18	123.00	31	0
	19	4661.00		
0	20	613.00		
	21	84.00		—
	22	4677.00		
2	23	636.00		
	24	145.00	55	0
Tf1	25	4185.00		
	26	767.00		
epicenter 4	27	140.00	48	0
	28	4586.00		
6	29	774.00		
	30	112.00	20	0
	31	4136.00		
8	32	887.00		
	33	111.00	19	0
	34	4202.00		
10	35	793.00		
	36	97.00	1	0
	37	1404.00		
	38	396.00		
Tf1 .02%	39	1801.00	1709	50 %
FrI	40	70.00		
	41	133.00		
0.2%	42	2536.00	2461	93 %
	43	27.00		
2%	44	208.00		
	45	2466.00	2374	91 %

Concluded no ds 3' epo activity is detected in either LTI or Epicenter projected Tf1 pol

To Pag No

Witnessed & Understood by me,	Date	Invented by	Dat
	5/15/95	Record d by	5-12-95

Adjust Tf1 storage buffer from Tag to Epicentre TF1 conditions

ge No.

	Epicentre Tf1 storage buffer	LTI Tag storage buffer	
ris HCl	50 mM pH 7.6 (pH at Room Temp based on Tele call to Epicentre Tech line)	20 mM pH 8	need to increase molarity from 50 mM to 20 mM and pH from 8 down to 7.6
aCl	100 mM	0	need to add 100 mM NaCl to LTI SB
lycerol	50%	"	LTI same as epicentre
TT	1 mM	"	
EDTA	0.1 mM	"	
ven 20/NP40	0.5% each	"	

Experiment

10 ml buffer G

1M Tris HCl	pH (room Temp)	
0	8.02	(expect 8.0)
5 λ	7.99	add 125 µl of 1M Tris HCl to 10 ml Tf1 4-30-95
20 λ	7.92	
20 λ	7.81	
20 λ	7.77	
20 λ	7.72	
20 λ	7.67	

Cf = 20 mM Tris (in SB)
+ 12.5 mM Tris HCl added
32.5 mM

will follow this exact procedure for 10 ml of Tf1 4-30-95
(P.8 4.33 u/λ ⇒ new Cf = 10.0 / 10.614 × 4.33 = 4.08 u/λ

add 182 µl 1M Tris pH 7.5 ⇒ Cf = 50 mM pH = 7.60 pH

Vf = 182 µl + 125 µl = 307 µl

plus 307 µl glycerol = 10.614 ml

V total plus 62 mg NaCl ⇒ Cf = 100 mM

mix end over end ✓ 30 60 min

To Page No.

d & Understood by me,	Date	Inv nt d by	Date
Solano	5/15/95	R cord d by	5-12-95

From Page No. _____ Follow p. 84,9

[A]

5x Cheng (no dNTPs)	200 µl	✓
H2O	637.9	✓
activated DNA 3.7 mg/ml	135.1	✓
dATG-C-TP 10 mM ea	5 µl	✓
32P-γ-dATP 10 mCi/ml	2 µl	
5-16-95 w/ date 3000 Ci/mmol		

Vf = 750 µl for 10 Rxns

Cheng 1X
- glycerol
- Tricine pH 9.0 20
- KOAc. 85 m
- DMSO 20
- MgOAc 1.05
- 0.5 mg/
- Cf 50 µm

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨

[A] 98.1 →

TF1 LTISB + Vent (5-16-95) 1.89.1 →

TF1 Epicenter SB + Vent (5-16-95) 2 →

TF1 Epicenter Enzyme + Vent (5-16-95) 1.95 µl →

Vf = 100 µl

68 °C, remove 10 µl to 5 µl 0.2M EDT (spot 10 µl on GFC), and remove 5 µl to 5 µl Kill solution with cold dATP (P84,9) and spot 2 µl on PEI (P84,

at 0, 10, 20, 40 min

Repeat on p. 40

Conclusion: There is no real good way to do this experiment because Vent is present at very low level compared to ∞ incorporation is saturated at levels where turnover is barely detected by PEI method. Will have to settle for DNA 3' exo QC assay for units: see P 26

To Page N

Witnessed & Understood by m,	Date	Invented by	Date
Dennis Doyle	6/9/95	Rec'rd by	5-11-95

Page No.

.247.00
3736.00
4880.00
6046.00
2170.00
3243.00
3897.00
5694.00
2774.00
3405.00
4090.00
5613.00
3451.00
3498.00
6034.00
3820.00)
2169.00
2604.00
2612.00
2580.00
2193.00
2755.00
2480.00
942.00
053.00
113.00
817.00
182.00

Turnover

29 1925.00
30 3149.00
31 2983.00
32 3230.00
33 1091.00
34 1772.00
35 3000.00
36 3722.00

Expenses

Turnover

pmol

1	68601.00	1487
2	118176.00	2562
3	155582.00	5372
4	95344.00	
5	125044.00	
6	138326.00	
7	93269.00	
8	119376.00	
9	167655.00	
10	93777.00	
11	116666.00	
12	131003.00	
13	109619.00	
14	126936.00	
15	143456.00	
16	90599.00	
17	103792.00	
18	162204.00	
19	96493.00	
20	124924.00	
21	162506.00	
22	91166.00	
23	125191.00	
24	167630.00	
25	84292.00	
26	127063.00	
27	153977.00	
28	2135.00	RKOD
29	975.00	BKOD
30	110991.00	
31	110539.00	

2λ
2λ

ave 110765 69.2 cm/pm

To Page No. _____

ed & Understood by me,

Date 5/9/65

Invented by

Recorded by

Date 5-16-65

Project No.______

Book No.______ TITLE S200 of Tne

Exhibit 90
Appl. No. 09/558,421

From Page No. __

*buffer "S"

			Cf
1M Tris HCl 7.5 pH	20 ml	✓	20 mM
0.5 M EDTA	0.2 ml	✓	0.1 mM
glycerol	80 ml	✓	8 %
β me	0.357	✓	5 mM
3M KCl	16.7 ml	✓	50 mM
	1 L		

poured 6 ml S200

~~wash~~ equilibrate at 1 col vol/hr
(= 0.1 ml/min) for 2 hr

load 120 μl of Tne 36 u/μl 5-7-95
(= 2% col vol) (4320 units total)
Load on S200

by gravity.

elute at 1 col vol/hr
~~into ~ 95 fr~~

collect 50 μl frns (30 sec/fr

span ~0.5 (??)
A

*note no detergent in buffer S
maybe this is why activity died after a few weeks at 4°C (see P53)

To Page

Witnessed & Understood by me, [signature] Date 6/9/95

Invented by [signature] Date 5-17-95

Recorded by

Project No.________
Book No.________ TITLE Assay 5200 fractions

From Page No.__

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
fr#	10	13	16	19	22	25	28	30	35	40	45	50	55	60	65	70

dilute 1/100 →
2 μl →

Top Rxn mix 48 →
pH 20,5 10', 74°

Frn			pmol	u/μl
10	1	9978.00	199	3.0
13	2	9430.00		
16	3	16574.00		
19	4	12045.00		
22	5	10155.00		
25	6	7256.00		2.2
28	7	6430.00		1.9
30	8	6091.00		1.8
35	9	3680.00		1.1
40	10	2746.00		0.82
45	11	2002.00		0.60
50	12	1476.00		0.44
55	13	1118.00		0.33
60	14	694.00		0.2
65	15	970.00		
70	16	603.00		
~~200~~	17 2x	120535.00	75 cpm/pmol	

Results from Top of P.25

fr			u/μl
3	1	3216.00	0.97
4	2	3990.00	1.2
5	3	5279.00	1.6
6	4	5148.00	1.55
7	5	6128.00	1.8
8	6	8950.00	2.7
9	7	11386.00	3.4
10	8	16350.00	4.9
11	9	14464.00	4.3
12	10	19127.00	5.8
13	11	23242.00	7.0
14	12	24609.00	7.4
15	13	25276.00	7.6
16	14	24319.00	7.3
17	15	23374.00	7.0
18	16	16929.00	5.1
19	17	14764.00	4.4
20	18	15075.00	4.5
21	19	13028.00	3.9
22	20	10781.00	3.2

To Pag

Witnessed & Understood by me,	Date	Inv nted by	Dat
DPolanp	6/9/95	[signature]	5-18-95
		R cord d by	

N

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	-

1/150

Results on P 24

1-95

unit assay on for 13-17 : pool 20 µl of each

13-17 1 2 3 4 5 6 7 8 9 10

100 2

200 2

400 2

800 2

1600 2

3200 2

7-95 3 u/l

1000 2

2000 2

4000 2

8000 2

BIOSIS

11	13	1	183.00
12	14	1	216.00
13	15	1	115777.77

2 Rxn mix 72%

10,740

well normalized 5200 pool 13-17 against 36 u/l value for 7-7-95 (see P 23) 100

pool 13-17 $\left(\frac{7.7}{70.7}\right)(36\ u/l)$ = 3.9 u/l

(3.9 u/l)(7 rxns)(5 µl/rxn) = 136.5 units removed.

			pmol	u/µl	
3	1	16254.83	33	5.1	
4	1	12439.50		7.8	
5	1	5518.00		6.9	ave 7.7 u/l
6	1	2909.00		7.5	
7	1	1741.00		8.7	
8	1	866.00			
9	1	17122.03	55	53.2	
10	1	10433.3	44	67	70.7 ave
11	1	5683.00	110	70	
12	1	3137.00	77	77	

(see P 13)

To Pag No.

d & Understood by m ,	Date	Invented by	Date
Blamp	6/9/95	R corded by	5-18-95 5-19-95

ig N .__

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

1 1/100

Results on p 24

9-95
unit assay on fr 13-17 : pool 20 µl of each

	1	2	3	4	5	6	7	8	9	10
1B-17			1	2	3	4	5	6	7	8	9	10
1/100			2							
1/200				2						
1/400					2					
1/800						2				
1/1600							2			
1/3200								2		

5-7-95 3 u/l
1/1000
1/2000
1/4000
1/8000

B1402.5	11	13	1	183.00
	12	14	1	216.00
2 Rxns min	13	15	1	115777.77

2 2 2 2 2

10,740

well normalized 5200
pool 13-17 against 36 u/l
value for Tue 5-9-95
(see p 13) 00

pool 13-17 (7.7 / 36 u/l) / 10.7
= 3.9 u/l

(3.9 u/l)(7 rxns)(5 µl/rxn)
1365 units received

			pmol	u/µl
3	1	16254.83	33	5.1
4	1	12439.50		7.8
5	1	5518.00		6.9
6	1	2909.00		7.5
7	1	1741.00		7.7
8	1	866.00		
9	1	17122.03	55	53.2
10	1	10433.33	44	9.1
11	1	5683.00	10	7.0
12	1	3137.00	27	7.7

ave 7.7 u/l

70.7 ave
equals only 3b (see p 13)

To Pag No.____

sed & Underst od by me,	Date	Invent d by	Date
Polanp	6/9/95	Record d by	5-18-95 5-19-95

Project No.
Book No. ______ TITLE 3' exo (QC) assay for Tf1 / vent

From Page No.__ (see P20 where turnover on PEI didn't work)

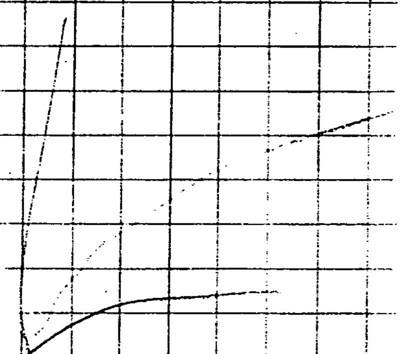
follow assay on p 11-17

actual units of Vent added based on 0.09 u vent/0.945 TA/u

	0 1 2 3 4 5 6		
Mix A P.16	4545 →		
	Tag 2 5		
Tf1/vent LTISB (5-16-95)	2		~0.2
1/10	2		.02
1/100	2		.002
1/1000	2		.0002
1/10 000	2		.00002
1/100 000	2		.000002

~~Tf1/vent Epicentre SB (5-16-95)~~
~~1/10~~
~~1/100~~
~~1/1000~~
~~1/10 000~~
~~1/100 000~~

~~Tf1/vent with epicentre Tf1 (5-16-95)~~
~~1/10~~
~~1/100~~
~~1/1000~~
~~1/10 000~~
~~1/100 000~~



H_2O 3 →

68°C, 60 min — start 2:35 pm

				-BKGD
BKGD →		88.00	21.31	
no dil	1	1684.00	4.87	1596
1/10	2	1323.00	5.49	1235
1/100	3	448.00	9.44	360
1/1000	4	143.00	16.71	55
1/10 000	5	96.00	20.39	8
1/100 000	6	101.00	19.90	13

Result: assay is not very l… in any range.
will assay at 1/10 and 1/100 in triplicate dilutions

T Page N

Witnessed & Understood by me,	Date	Invented by	Date
D Polonp	6/9/95	[signature]	5-22-95
		Recorded by	

[redacted]

Exhibit **94**
Appl. No. 09/558,421

Project No._______ Benetta
Book No._______ **TITLE** 2 μl of enzyme dilution

From Page No.__

5 min avg of Triplicates

Front middle ori	413.00	4406				282.00		avg
	441.00		% lost from ori		Vent	681.00		
	3550.00		minus BKGD			3216.00		
I SB	345.00	4050				252.00		3193 ori
	557.00		314	244		623.00		
	3148.00		520	199		3170.00	5 min	
			3275	-755	19%			
	185.00	3874				85.00		
	562.00				BKGD	325.00	4177	
	3127.00					3767.00		
duplicate SB	308.00	4410			BKGD	60.00		
	524.00					317.00	4669	
	3578.00					4292.00		
	272.00	4216				469.00		
	603.00		302	229		271.00		
	3341.00		532	231		4391.00		
			3434	-596	15%			
	325.00							
	528.00							
	3383.00							
duplicate Tf1	412.00							
	509.00							
	2885.00							
	453.00		410	387				
	515.00		486	145				
	3004.00		2995	-1035	26%			
	515.00		(4030 - 2995) / 4030					
	434.00							
	3096.00							

To Pag N

Witn ss d & Und rst d by me, [signature]

Date 6/22/95

Inv nted by [signature]

R c rded by

Dat 5-23-95

g N .__

		TIME		total sum	% lost from ori
1	front	324.00			
2	middle	726.00	1.00		
3	ori	1780.00	1.00	2830	
4	ave	348.00	1.00		
5		960.00	1.00	3117	
6		1809.00	1.00		
7		458.00	1.00		
8		830.00	1.00		
9	1764	1703.00	1.00	2991	43%
10		483.00	1.00		
11		608.00	1.00		
12		1726.00	1.00	2817	
13		477.00	1.00		
14		515.00	1.00		
15		1698.00	1.00	2690	
16		288.00	1.00		
17		856.00	1.00		46%
18	1658	1670.00	1.00		
19		729.00	1.00		
20		627.00	1.00		
21		1452.00	1.00		
22		632.00	1.00		
23		511.00	1.00		57%
24		1199.00	1.00		
25		471.00	1.00		
26		686.00	1.00		
27	1354	1410.00	1.00		
28		374.00	1.00		
29		664.00	1.00		
30		1398.00	1.00		50%
31		200.00	1.00		
32		786.00	1.00		
33		1641.00	1.00		
34		128.00	1.00		
35		947.00	1.00		
36	1573	1682.00	1.00		
37		97.00	1.00		
38		477.00	1.00		
39		3007.00	1.00	3581	
40		66.00	1.00		
41		591.00	1.00		
42		3146.00	1.00	3803	
43		60.00	1.00		
44		480.00	1.00		
45	3122	3214.00	1.00	3754	
			1.00		

Result. The sum of all counts when enzyme is present (ave 2879) is less than for the no enzyme blank (ave 3713 ≈ 22% of counts unaccounted for.

43% in each case ≈ 1500 CPM is lost from origin (i.e. ^3H removed by exo) but ≈ ½ of that appears in front and middle. Also, why does any appear in middle it looks like ^3HMP partly missing its middle.

Since ^3H runs in front it may be quenched by DNA contaminants that smear PET at the front.

% label removed from ds DNA substrate

	2λ enzyme mix	5λ enzyme mix
CTI SB	19%	43%
Epicentre SB	15	46
Epicentre TfI	26	57
Vent pol	21	50

To Page No.____

d & Understood by me,

Date 6/9/95

Invented by

Recorded by

Date 5-24-95

Project No.
Book No.
TITLE Pol unit assay for Nira TfI/Vent m
same as P20 except need less units
for in assay range & use
" as for Taq unit assay P12,9

From Page No.

TfI Chem unit assay mix

H_2O	1,056 ml	✓✓✓	35 Rxns
5X Chem buffer (see P20)	350 λ	✓✓✓	
activated DNA 3.7 mg/ml	236 λ	✓✓✓	(0.5 mg/ml)
dATCG-TP 10 mM ea	35 λ	✓✓✓	200 μM each)
^{32}P α dCTP 10 mCi/ml	3.5 ul λ	✓	
	1.68 ml		use 48 μl/Rxn

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
TfI Chem unit assay mix	48 μl →																							
used "TfI 4" ie #4 working stock																								
TfI 4.33 u/λ (P8)																								
1/250	2				2																			
1/500		2				2																		
1/1000			2				2																	
1/2000				2				2																
TfI/Vent 5/16/95 LTISB (~1u/λ TfI using Epicentre units; its ~4u/λ using my units (P.8) for TfI)																								
1/250									2					2										
1/500										2					2									
1/1000											2					2								
1/2000												2					2							
TfI vent 5/16/95 Epicentre SB																								
1/250																		2				2		
1/500																			2				2	
1/1000																				2				2
1/2000																					2			2
TfI/Vent 5/16/95 (Epicentre TfI)																								
1/250																								
1/500																								
1/1000																								
1/2000																								

} using duplicate serial dilutions for each set

~~[illegible]~~ 74°C, 10'

dilute by adding 2λ enzyme to 498 μl Taq dil buffer for

T Pag

Witnessed & Understood by me, [signature] Date 6/9/95 Invented by [signature] Date 5-24-95 Recorded by

g N .__

, 30 31 32 33

	SAM	CPM1	(-BKGD) pmol	u/µl
Tf1	1	4165.00	127	4.77 (was 4.33 u/λ on P.8)
	2	2395.00		5.14
	3	1575.00		6.22
	4	1018.00		6.9
	5	4108.00		4.70
	6	3019.00		6.7
	7	2232.00		9.5
	8	1205.00		7.7
LT II SB	9	3788.00		4.5
	10	2354.00		5.0
	11	1425.00		5.47
	12	1090.00		7.62
	13	4135.00		4.7
	14	2353.00		5.0
	15	1688.00		6.78
	16	1095.00		7.67
Epicentr SB	17	3543.00		4.00
	18	2336.00		5.00
	19	1431.00		5.51
	20	1040.00		7.13
	21	3388.00		3.81
	22	2191.00		4.64
	23	1354.00		5.12
	24	857.00		5.31
Epicentr Tf1	25	3746.00		
	26	2188.00		
	27	1548.00		
	28	866.00		
	29	4053.00		
	30	2474.00	71	5.3
	31	1456.00		
	32	905.00		
BKGD	33	322.00		
2λ Rx mix	34	72445.00	45.3 cpm/pmol	

Z
Z
Z —

will repeat this with 5 duplicates of the 1/250 µl dil for each on P.47

To Page No.____

d & Understood by me,	Date	Invented by	Date
[signature]	6/9/95	[signature]	5-24-95
		R corded by	

Exhibit **96**
Appl. No. 09/558,421

34 Project No.______ Book No.______ TITLE new, B and M 2X mix

assay samples P121,9

cmts: 1.1x field test ("old")

From Page No. ∅

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
rTaq 1-31-95 5u/µl																									
1/125	2			2																					
1/250		2			2																				
1/500			2			2																			
1.1X new (may 8, 1995																									
no dil							2			2															
1/2								2			2														
1/4									2			2													
1.1x old																									
no dil													2			2									
1/2														2			2								
1/4															2			2							
BM 2X																									
1/2																			2			2			
1/4																				2			2		
1/8																					2			2	–

Taq rxn mix (P120,9) 48 µl → 50 µl   74°C, 10'

for rTaq/125 and 2 µl undiluted of sample

see circled #a P35

$$\frac{\text{new } 6506 + 7030}{\text{rTaq } 10692 + 12403}\ (.04\ u/\text{rTaq lysed}) = .025$$

$$\frac{6729 + 6660}{10692 + 12403}\quad .023$$

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
Polanyi [signature]	6/9/95	[signature]	5-25-95
		Recorded by	

g No.__

			pmol	u/µl			
1/125	1	10692.00	417	7.7			
1/125	2	5333.00		7.7			
1/125	3	3112.00		8.0	← (-3338 BKGD)		
	4	12403.00		8.9			
	5	6387.00		9.2	Total units in	* in 50 µl at 1X	average
	6	3853.00		10.0	in 50 µl at 1.1X	1.71	* 1.76
modif	7	6505.00	250	.038 u/µl	1.88 u		
1/2	8	4314.00		.050	2.50	2.27	
1/4	9	2381.00		.047	2.39	2.17	2.22
	10	7000.00		.040	2.0	1.82	
	11	4401.00		.051	2.5	2.27	
	12	2364.00		.047	2.4	2.1	* 1.75
	13	6729.00		.039	1.95	1.77	
	14	3962.00		.046	2.3		
	15	2247.00		.045	2.2		1.88
	16	6660.00		.038	1.90	1.73	
	17	3659.00		.038	1.9		
	18	1940.00		.037	1.9		
1/2	19	3456.00		.036	.87		
1/4	20	1705.00		.032	.77		0.86
1/8	21	1368.00		.047			
1/2	22	3028.00	117	.051	.84		
1/4	23	2005.00		.059	.94		
1/8	24	900.00		.026			
	25	333.00	BKGD				
	26	62215.00	37.9 cpm/pmol				

7.7 is a reflect since this "5 u/µl" is normalized to amplitude that was ~8 u/µl

red is average
tal units in 50 µl
of 1X for the assays of
undiluted/µl of 1.1X mix
e use 1.76 units for "new"
d 1.75 units for old "field test"
as my first time points for these samples. (note: appeal ~2
nd dilutions above indicate 2.22 u and 1.89 u for old and new)
see next assay on P52 which is 1 month time point
using 2 µl undiluted.

* mix is 1.1 X
so at 1X mix is
(u/µl in 1.1X)

To Page No.____

ed & Understood by me,	Date	Invented by	Dat
Polano	6/9/95	Record d by	12 5 95

Exhibit 97
Appl. No. 09/558,421

Project No.________
36 Book No.________ TITLE unit assay for stability assay of 1.1X mix (p121, 9) (at [illegible]

From Page No.__

Group	#	Value	ave u/μl	% of p.122 (zero time pt)	Notes
1	1	8878.00			
	2	9039.00	.036	98	
	3	9623.00			
2	4	8220.00			
	5	8228.00	.032	98	
	6	8109.00			
3	7	8855.00			
	8	8307.00	.034	106	
	9	8584.00			
4	10	6857.00			
	11	7096.00	.027	93	
	12	6660.00			see [illegible] results on p[illegible]
5	13	9295.00			
	14	8535.00	.035	105	
	15	8519.00			
6	16	6780.00			
	17	5930.00	.026	98	
	18	5879.00			
7	19	8250.00			
	20	8545.00	.034	104	
	21	9288.00			
8	22	8605.00			
	23	7590.00	.032	91	
	24	7975.00			
9	25	7909.00			
	26	7993.00	.031	99	
	27	7301.00			
10 (not [illegible])	28	2863.00	.012 u/μl		
	29	3151.00	data from p 37, 38 → .015		← (repeat [illegible] p 153)
	30	3188.00			
11	31	7926.00			
	32	7626.00	.030	94	
	33	7355.00			
12	34	8180.00			
	35	8930.00	.034	100	
	36	9000.00			
13	37	2662.00			
	38	2885.00	.011	35	← (will repeat)
	39	2632.00			
14	40	8091.00			
	41	7872.00	.031	89	
	42	7722.00			
15	43	7664.00			
	44	7828.00	.031	97	
	45	8063.00			
rTaq	46	10091.00			
	47	9701.00			
	48	10062.00	10117 ave ⇒ 42 bpmol ⇒ 7.99 units/[illegible]		
	49	10476.00	(expect [illegible] since 5u/[illegible] EKBT[illegible] normalized to amplitaq of [illegible])		
	50	10230.00			
	51	101.00			
	52	56411.00	35.6 cpm/pmol		
	53	57488.00			

To Page No.

Witnessed & Understood by me, [signature]
Date 6/9/95
Invented by [signature]
Recorded by
Date 5-26-95 5-26-95 [initials]

g No.__

0 time on P.154, 9 3-13-95
1 month P.174, 9 4-11-95

assay same as P.121, 9 for 4°C stability study
used same assay mix as P 5-25-95

	Reaction Tube #	µl enzyme Rxn	1% to Tween 20 NP40	Tag mix assay mix (P.121,5)
	1-3	2		48 µl
	4-6			
	7-9			
	10-12			
	13-15			
	16-17			
	18-21			
	22-24			
	25-27			
	28-30		0.5λ	
	31-34	3.64		
dil 1/2.5	34-36	2		
	37-39			
	40-42			
	43-45			
Stability				
	46 ~~47~~ 47		0.5λ	
	48 ~~51~~ 49			
5 µl/µl 1-31-95	~~52-56~~			
dil **	50-52			
	53 2λ of "old mix"			
	54 2λ of second mix			

74°C, 10
kill with 10µl
0.5M EDTA
spot ~~40~~ 30 µl
on GFC

10% TCA, 1% NaPP
5'
↓
3 × 5' in 5% TC
1 × 5' in 95% ETO

current ^{32}P mix

µl Tag dil buffer + 5µl sample #12
5λ + 620λ dil buffer

To Page No.____

sed & Understo d by me, Date 6/9/95 Invent d by Date 5-30-95
Record d by

[redacted]

Project No.________ at zero %

Book No.________ TITLE time remaining (P174.9)

From I			µCi/µl	time	2 months	1 month
1	1	3864.00	.024	.037	65 %	89
	2	4350.00				
	3	3113.00				
2	4	155.00	0.00090	0.033	2.7%	106
	5	130.00				
	6	133.00				
3	7	4121.00	0.029	0.032	91%	106
	8	4864.00				
	9	4225.00				
4	10	3184.00	0.021	0.029	72%	93
	11	3267.00				
	12	3240.00				
5	13	3952.00	0.025	0.033	76%	91
	14	3728.00				
	15	3817.00				
6	16	2585.00	0.017	0.027	63%	78
	17	2492.00				
	18	2795.00				
7	19	4009.00	0.028	0.033	85%	84
	20	4530.00				
	21	4334.00				
8	22	4199.00	0.028	0.031	90%	89
	23	4281.00				
	24	4326.00				
9	25	4248.00	0.026	0.031	84%	90
	26	3739.00				
	27	3922.00				
10	28	65.00	0.00053	0.022	0 ~~2.4%~~	0
	29	86.00				
	30	93.00				
11	31	4340.00	0.026	0.032	81%	78
	32	3827.00				
	33	4038.00				
12	34	5155.00	0.035	0.034	103%	—
	35	5666.00				
	36	5301.00				
13	37	3927.00	0.026	0.031	84%	97
	38	3981.00				
	39	4352.00				
14	40	4214.00	0.027	0.035	77%	83
	41	3962.00				
	42	4287.00				
15	43	3992.00	0.026	0.032	81%	106
	44	3958.00				
	45	4278.00				
-40°C ct	46	2014.00	0.013			
	47	2016.00				
4°C	48	1504.00	0.0098			
	49	1509.00				
Tag	50	5941.00	(.04 µCi/µl) by definition 6177			
	51	6122.00				
	52	6468.00				
old	53	49708.00	46819			
new	54	40962.00				

same as p 36 when corrected for 30µl on GFC instead of 40µl and 4 day decay of ^{32}P

T Pag N

Witnessed & Understood by me,	Date	Invented by	Dat
[signature]	6/9/95	[signature]	5-30-95
		Recorded by	

g N .

sp act

$\frac{\text{46819 cpm}}{\text{100 pmol dCTP) (4)}} = 29.3 \text{ cpm/pmol(nt) DN}$

$\left(\frac{\text{6177 avg Taq cpm}}{\text{29.3 cpm/pmol}}\right)\left(\frac{60\lambda}{30\lambda}\right) = \boxed{\text{421 pmol DNA synthesis}}$

1 unit 10 n mol / 30' at 74°C

$\left(\frac{.421 \text{ n mol}}{10 \text{ mol}}\right)\left(\frac{30'}{10'}\right) = 7.89 \text{ u/µl}$

$\frac{\text{.04 u/µl in Rxn}}{\text{7.89 u/µl for Taq}}\left(\frac{\text{sample cpm}}{\text{Taq cpm}}\right)$ = µl of sample

100 µM each dNTP

To Page No.

ed & Understood by m ,	Date	Invented by	Date
Polanp	6/9/95	R corded by	5-30-95

Project No.
Book No.
TITLE Repeat of P. 20 : stability of Tfl / Vent mixes.

From Page No.

	[A]
H_2O	446.4 ✓
5X Cheng buffer (no dNTPs)	140 µl ✓
activated DNA 3.7 mg/ml	94.6 µl ✓
dA,T,G,C-TP 10mM each	3.5 µl ✓
α32P dATP 10 mCi/ml (ref 6-2-95) 3000 Ci/mmol	1.5 µl
	686

Cf=

tube #	1-4	5-8	9-12	13-16	17-20	21-24	25-28	29-32
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)

[A] 98 µl → (Vent diluted) →

u/µl Tfl in epicentre units (its ~3.5 units really) and .09 units Vent/µl

Tfl LTISB + Vent (5-16-95) 2 to 7 →

2 → 1X Cheng

— (no enzyme)

← 0.18 units Vent in...

Tfl Epicentre SB + Vent 5-16-95 2 →

mistake : this is 0.09 units / 100 µl. ... be 0.18 - ie Vent used per 50 µl PCR * Vent ...

Tfl Epicentre enzyme + Vent (5-16-95) 2 (15) →

2 µl .045 u/µl Vent diluted in 1X Cheng buffer *

2 µl of Vent ... 2 u/µl of 2-... in 86.9 µl 1X Cheng ... (lot # 17)

Vf ~ 100 µl

68°C

start with addition of enzyme to preheated mix.
remove 10 µl to 5 µl 0.2 M EDTA (spot 10 µl on GF...
and 5 µl to 5 µl Killing solution with cold dAM...
(spot 2) on PEI at 0, 5, 10, 15, 20 min.

resolve in 1M LiCl

T Pag N

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/9/95	[signature]	5-31-95
		Recorded by	

N

cpm − background cpm / specific activity = pmoles

pmoles / ave pmoles incorporated = % turnover — should be constant; 2 replicates of each time pt.

AM		CPM1 / turnover		
1	5'	885.00	ex 885−543 / 103.31 = 3.31	0.042
2	10'	1241.00	4.76	0.069
3	15'	1074.00	5.14	0.065
4	20'	1269.00	7.03	0.062
5		984.00	4.27	
6		1332.00	7.64	
7		1678.00	11.0	
8		1590.00	10.1	
9		830.00	2.78	
0		1213.00	4.49	0.021
1		1195.00	6.31	0.066
2		1460.00	8.38	0.055
3		555.00	0.116	0.062
4		1228.00	6.63	
5		1225.00	6.60	
6		1425.00	8.54	
7		764.00	2.14	
8		977.00	4.20	
		CPM3		0.039
19		1212.00	6.48	0.028
20		1453.00	8.51	0.043
21		895.00	3.41	0.061
22		772.00	2.22	
23		1009.00	4.51	
24		1365.00	7.96	
25		746.00	1.96	0.70
26		438.00	−1.02 X	0.47
27		757.00	2.07	0.14
28		609.00	0.04	
29		412.00		
30		578.00	x̄ 543	
31		488.00	background,	
32		693.00	no enzyme	

> 1 replicate/time pt

result: 1) turnover began to reach a plateau by 10 min. We expected turnover to continue increasing over time after DNA synthesis stopped.
- Tfl completes w/ Vent at nick

2) turnover by Vent alone in Cheng buffer is lower than expected. 25% turnover was observed in another experiment.
453 pmole in 20 min / 25 = 1/25 syn by vent
The high signal to noise level for the Vent samples makes it difficult to say what the turnover is. Turnover is higher when Vent is mixed w/ Tfl.
- Tfl creates mismatches that are targets for Vent exo

do: repeat w/ 1, 2, 3, 4, 5 min time points and more Vent enzyme in the Vent alone samples, use new PEI plates

specific activity: cpm of 2ul spot of mix A (2 replicas) 36438 + 46210 x̄ = 41,324 cpm

$$\frac{\left(\frac{100\text{ul rxn}}{2\text{ul spot}}\right)\left(41324\text{ cpm}\right)}{\left(5000\ \frac{\text{pmole}}{\text{nt}}\right)\left(4\text{ bases}\right)} = 103.31\ \frac{\text{cpm}}{\text{pmole (nt) DNA}}$$

50 uM each dNTP in 100ul rxn
50 umole/L x 100 x 10⁻⁶ L = 0.005 umole = 5 nmole = 5000 pmole

To Pag No.

d & Understo d by me,	Date	Inv nted by	Date
Polanyi	4/9/93	Recorded by Carolyn Combs	5-16-93

From Page No.

$$\frac{\text{sample cpm}}{\text{specific activity}} \times \frac{100\,\mu l\ rxn}{10\,\mu l\ spot} \times \frac{15}{10}\ \text{dilution}$$

		Incorporation	pmoles
1	1	49596.00	49596 / 103.3 x 15 = 7,201
	2	74066.00	10,754
	3	88521.00	12,853
	4	95661.00	13,889
2	5	50395.00	7,317
	6	69543.00	10,097
	7	82738.00	12,013
	8	93515.00	13,578
3	9	45114.00	6,550
	10	64768.00	9,404
	11	81250.00	11,797
	12	96711.00	14,042
4	13	49095.00	7,128
	14	71796.00	10,424
	15	81335.00	11,809
	16	95798.00	13,909
5	17	50290.00	7,302
	18	70938.00	10,230
	19	88754.00	12,887
	20	98147.00	14,250
6	21	48881.00	7,097
	22	85245.00	12,377
	23	85694.00	12,442
	24	91420.00	13,274
7	25	1932.00	281
	26	2581.00	375
	27	3000.00	436
	28	3120.00	453
8	29	854.00	123
	30	777.00	113
	31	183.00	26.6 no enzyme
	32	487.00	70.7 no enzyme
		~~9.00~~	
		~~6.00~~	
2ul mix A =		36438.00	$\bar{x}$ 41,324 cpm
		46210.00	

for calculation of specific activity

103 cpm/pmol

Synthesis ~~first~~ was almost complete by 10min
By 20min ~14nmoles of the 20nmole had been incorporated - hi, in the 1st

To Page N

Witnessed & Understood by me,	Date	Invented by	Date
	6/9/95	Recorded by	5-31-95

EKBT1
323 u/μl (P91, see 9)

157.2 μl

Tog storage buffer
(Price dilutent)

$V_f = 10 \cdot \frac{157}{ml}$ ml

10 ml

$(C_f = 5\ u/\lambda)$

mix end over end 1 hour

storage buffer is from 12-7-94
with Price dilutents

ed & Understood by me,
Polanyo

Date 6/9/95

Invented by

Recorded by

Dat 5-31-95

Exhibit 101
Appl. No. 09/558,421

Project N

44 Book No.____ TITLE primer degradation using Tf1 (can see P 17, the section #11)

From Pag No.__

	(1)	(2)	(3)	(4)	(5)	(6)
5X Cherry (no dNTPs)	20 µl	→	→	→	→	→
32P "33 mer correct" same as (P12 and 14) 5 µM	4	→	→	→	→	→
Tf1/Vent 5-16-95 CTI 5B	10	—	—	—	—	—
Tf1/Vent 5-16-95 Epicentre 5B		10				
Tf1/Vent 5-16-95 Epicentre Tf1			10			
Tf1 LTI 4.33 u/µl (P.8)				10		
Vent .09 u/µl lot #17 (opened 2-24-95) LTI 5B P.6 (same stock as in Tf1 P.8)				10		
H2O	66	→	→	→	→	→
	Vf = 100 µl					

(0.9 units va in 10 µl re

* 2 u/µl Vent diluted into CTI Taq 5B
2 µl Vent
42.4 µl Taq 5B
44.4 Vf

68°C. remove 10 µl to 5 µl agile rxn
stop rxn at 0, 5, 10, 20, 40, 60, 80, 100 m

● add enzyme on ice → take 0 time point, start timing when thin walled tube put in preheated 96° at 68°C

16 hrs PAGE ~44 watts (Volts range from 160
get ~ 12 cm/hr for BPB

T Page N

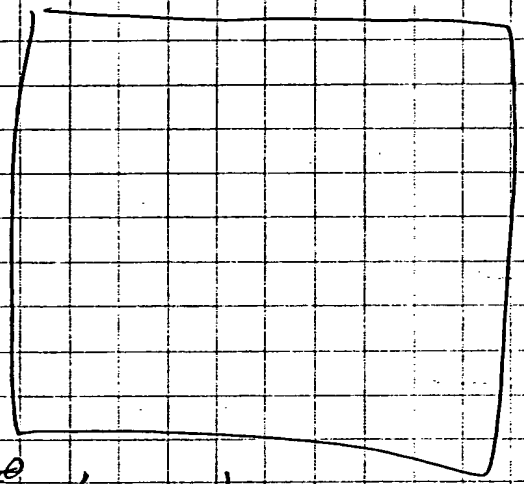
Witness d & Understood by me,	Date	Invented by	Dat
[signature]	6/9/95	Recorded by	6-2-95

ag N .__

32P-33mer is ~ 10x10^6 CPM/µl
↓ dilute 1/1000

1.5 x 10^-6 M
5' end labeled w/ 23mer ~ 20 mM nt
5µl H2O
5µl

10mM dATP stock



comm stock
1:5 dilution
1 vol stock, 4 vol H2O TE
M ATP
M dATP

M 23mer
eled

-3mer
1/1000

To Pag No.____

sed & Understood by me,	Date 6/9/95	Inv nted by	Date 6-2-95
Doolan		R cord d by	

[redacted]

Project No._______
Book No._______ TITLE ________________

From Page No.__ Results:

1. ice does not shut down 3' exo (start with Mg next (?))

2. there is very rapid loss of first 1-10 nts, then very slow degradation i.e. with time, need less enzyme and/or shorter time points

3. No apparent endo or 5' exo activity for Tfl alone as seen on P14. Here its 5λ Tfl (4.33 u/λ) per 50 μl compared to 2λ on P14 but still should see plenty of loss of full length here based on rate seen on P14. (also was by BW on P14 / Try ± dNTPs) also P14 has dNTPs present but not here. Maybe be some kind of primer extension involved in loss of full length primer — i.e. extension to many lengths contributes to apparent loss of primer or to production of a 5' exo target — could be primer dimer extension or hairpin within 33 mer.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/9/95	[signature]	6-2-95
		Recorded by	

ıg N .__

95 - 6/6/95

pose: To measure 3'→5' exonuclease activity of TFI/Vent using the primer degradation assay.

ckground: An earlier trial of this experiment (NB 10 page) was done by removing aliquots of the rxn at time points 0, 5, 10, 20, 40, 60, 80, 100 min. The primer was degraded almost to the maximum amount by 10 min. Since we want do determine the 3'-5' exo activity rate, we need to find the linear range of the assay. This can be done by taking shorter time points or by taking a single time point on a series of enzyme dilutions (doubling [enzyme] should double extent of degradation in the linear range of the assay) We'll do this trial expt w/ just 1 enzyme sample - TFI/Vent in LTI SB - and 13 different dilutions. Once the linear range is found, we can repeat the exp. just on that range.

terials: ^{32}P γATP for end labeling primer
primer = 33mer correct
Taq dilution buffer - cc aliquot
LTI storage buffer - RL aliquot
TFI/Vent enzyme mix - from stability study
9600 PCR machine & tubes
5x Cheng buffer - cc aliquot
PNK = T4 kinase & buffer - ~~old~~ - LTI new
8% sequencing gel & buffer - LTI premade
Stop buffer
sterile H_2O

d & Understood by me, Polaroid

Date 6/9/95

Invent d by [signature]

Recorded by Carolyn Combs

Date 6/9/95

Project No. _____

2 Book No. _____ TITLE _____

From Page No. ___

Procedure

6/5 ① end-label the 33-mer primer w/ ^{32}P γ ATP = Kinase reaction

reference = notebook 1C p 12

mix:	H_2O sterile	———	25ul ✓	+ 25ul
	5x Kinase buffer		12ul ✓	+ 12ul
20uM	33mer ~~constant~~ correct	p 133 20 pmole/ul	15ul ✓	+ 15ul
AC4521	^{32}P γ ATP ~~10mCi/ml~~ ref		5ul ✓	+ 5ul
	PNK 10u/ul new	FE54/9	3ul	+ 3ul
			60ul	/ 120ul

* added after 1st 5 co were added

incubate 37°C, 30min ✓

~55°C, 5min ✓

store labeled DNA + unincorporated label at -20°C — run some ol

6/5 ② make a ~~4~~ 8% denaturing-sequencing gel + buffer → both premade by

- 1,75ml bottle of 8% mix (cold room) + 450ul 10% AP (made fresh)

  0.0868g AP ←

  868ul H_2O ⇒ $\frac{.0868g}{.868ml}$

- after pouring gel, shake up remaining gel mix so it can be used to fill leaks, ect.

- store gel upright, ON, at RT w/ H_2O-soaked towels and saran ✓

T Pag N

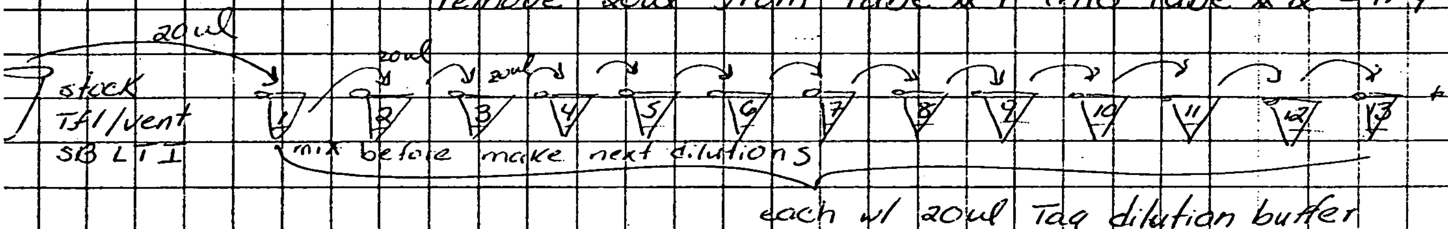
Witn ss d & Underst d by m ,	Dat	Invent d by-	Dat
	6/9/95		6/9/95
		R c rded by	

ıg N .__

…t up enzyme reactions , ~~14 rxns~~ for 16 rxns:
89.6ul H_2O ✓
6.4ul ^{32}P 33 ✓
32ul, 5x Cheng ✓
128ul

…laster mix A = ~~87~~ ul H_2O
…r rxn ⇒ 5ul H_2O, 0.4ul ^{32}P 33-mer, 2ul 5x Cheng, 8ul
3 ul ^{32}P labeled 33-mer from 6/5
30 ul 5X Cheng buffer
120ul , enough for 15 rxns
store on ice

…nzyme dilutions : Tfl/vent in SB LTI diluted in Taq dilution buffer
- 20ul Taq dilution buffer in tubes 1-13, big tubes
- add 20ul Tfl/Vent to tube #1 = 1:2
- ~~vortex~~ flick to mix and store on ice
- remove 20ul from tube #1 into tube #2 = 1:4 ect



- store dilutions on ice

…dd 8ul Mix A to 9600 tubes # 1-14 . * radioactive and prewarm to 68°C in 9600 machine

Tube #	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mix A 8ul	equilibrated to 68°C →													
…ted Tfl/vent 2ul	→													no enzy 2ul Taq dil buffer
…tion factor = SB LTI	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	

add enz. to tube, flick to mix, stagger rxns by 1min

…er 15min, remove tube and add 5ul stop - pipet up + down 3X and Keep on ice until gel is run

…gel, run 15, 1-15 ~~14, 1-13, 14~~ (3ul)
~~old labeled ^{32}P labeled 33-mer from 6/1/95~~
~~2ul old ^{32}P-33, 12ul H_2O~~ remove 2ul, 2ul 5x Cheng, 6ul H_2O or more label
2ul old + 10ul H_2O
- load
1:6 2ul 2ul 5x Cheng, 30ul H_2O 4ul, 5ul stop 3ul
3ul, 300ul dil, 2ul 1/598ul H_2O, 3ul load 3ul

…at before loading, 95°C 5min in 9600
…in down 11:20am - 1700 v ⇒ 65w

To Page No.______

…ed & Understood by me, [signature] Date 6/9/95 Invented by [signature] 6/9/95 Date 6/9/95
Recorded by Carolyn [signature]

Project No._____

Book No._____ TITLE _____

From Page No.__

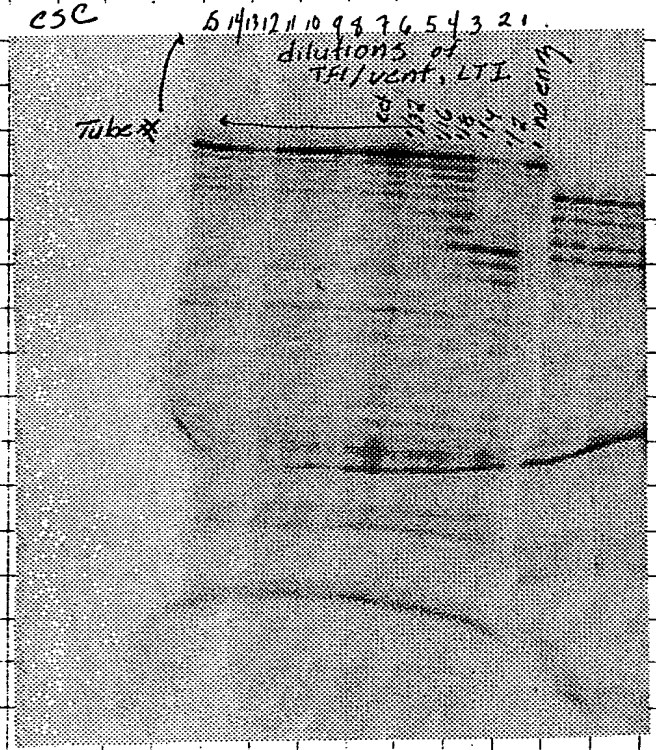
- BQP ran down to bottom glass clamp on gel rig, ~1hr, 1700 V (constant), ~65W
- transfer to drain buffer Whatman paper,
- cover w/ saran & cut to size
- dry - 2 pieces whatman under gel, saran over gel, dry ice in trap
- set vacuum & heat for 1hr = 1-12:45-1:45 PM
- set in phosphoimager cassette - bottom to bottom w/ saran ~2 PM for ON exposure

Result: ON exposure on phosphoimager

csc

C:\DATA\CC.GEL 1995:06:07 07:57:48, Range = 0.11-10000.00 Counts, 0.50x

csc



Conclusion: The 1/4, 1/8, 1/16 dilu
~~gave~~ span the linear range
the primer degradation
primer length
time
Now, we'll do a co
time pts of each
dilution to gather
better data from the lin
range. The data w/
used to show 3-5 exp ac
for stability study.

T Pag No

Witn ssed & Understood by m ,	Dat	Inv nt d by	Dat
DDPolanco	6/9/95	6-9-95	6/9/95
		Rec rded by Carlos Combs	

Determination of 33-mer primer mobility on PEI plate

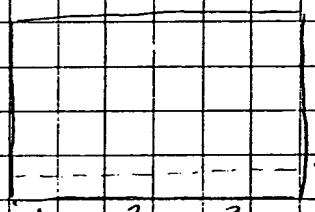
Page No. — 6/6/95

Purpose: To determine the relative mobility of the ^{32}P-33mer correct primer on a PEI plate, developed in LiCl.

Background: Originally we wanted to determine the specific activity of the ^{32}P-33mer primer that was used in the primer degradation assay w/ TFI/Vent (NB 10 p) However, we later decided that it is not important to find the specific activity since we can do a no enz. control each time the assay is done. Now we want to determine the mobility because we observed that the cold oligo did not run as expected on the PEI plate, and we just are curious about ~~how~~ where the oligo ran.

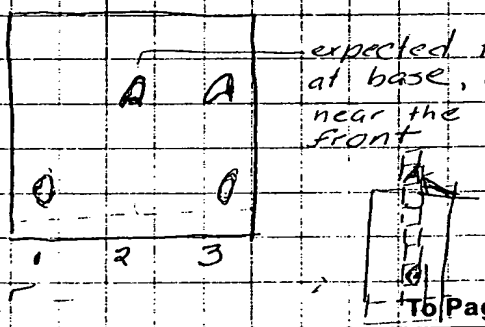
Materials: cold 33-mer correct
^{32}P 33mer correct - labeled on 6/9/95
ATP
PEI plate
1M LiCl
scint vials + cocktail

Procedure: > spot on PEI plate →



	1	2	3
20mM ATP	2ul		2ul
20mM 33mer		2ul	2ul
1/1000 ^{32}P-33mer			2ul

> set plate in developing chamber w/ 1M LiCl ~1hr - ran 1/2 way
> circle control spots (ATP + cold 33mer) in lane 3 under UV light → sketch of how plate looked



γATP ^{32}*
ADP
^{32}P-33mer*
cold 33mer
pp$_i$ ^{32}*

> cut lane 3 into 8 pieces and count in scintillation counter -

To Page No.

Read & Understood by me, Polanyi — Date 6/9/95

Invented by — 6-12-95 — Date 6/9/95

Recorded by

From Page No.

C.S.C

Result:

PAGE:

USER: 1 ID:32P 1.0 CPM PRESET TIME: 1.00 TUE 06 JUN 1995 15:36
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 0 ADC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL:1000 2SIGMA: 0.05 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
HALF LIFE(DAYS):N

SAM	CPM1		TIME	ER
1	111.00	- baseline	1.00	
2	307.00	- area around where cold ATP standard ran	1.00	
3	136.00		1.00	
4	117.00		1.00	
5	60.00		1.00	⇒ primer runs like dUMP
6	215.00	- area around where cold 33mer primer ran	1.00	
7	27.00	- near solvent front	1.00	

The labeled 33-mer primer ran like the dAMP runs on a PEI plate, up near the solvent front, not at the origen.

This result was expected from the information we heard from the chemistry group: DNA stays at the origen because of its large size.

oligonucleotides run like dUMP because they have same charge:mass ratio as dUMP

To Pag

Witness d & Und rsto d by m , Dat 6/9/95

Invent d by 6-12-95 Dat 6/9/95

R c rded by Pauline Ponde

Tfl/Vent primer degradation assay - time course on serial dilutions for a time point of stability study

Purpose: To measure 3'-5' exonuclease activity of Tfl/Vent mixes in 3 buffers at the zero time point of stability study

Background: Linear range of assay determined in previous expt, NB II page 1 - dilutions 1/4, 1/8, 1/16 looked good (↳ = 0.045 units)
- linear range
- now do time course of these dilutions

1/4 dilution ⇒ 0.09 u/ul / 4 x 2ul = 0.045 units in 10ul reactions; 1/8 dil ⇒ 0.0225 units; 1/16 ⇒ 0.0113 u

Materials: ^{32}P 33mer correct - labeled on 6/5/95
Tfl/Vent in LTI, epicenter SB, epicenter Tfl
8% gel
mix A

* Vent dilution 2000 u/mL
2ul vent Lot 17 2/24/95
42.4ul Taq 513
44.4 ul at 0.09 u/ul
22.2x dil

Procedure:
1) make mix A, enough for ~~14~~ 15 rxns - 90ul per rxn

per rxn ~~5.0ul x 14~~ = 20ul x ~~14~~ 15 = ~~280~~ 300ul 5X Cheng ✓
4ul x 14 = ~~56~~ 60ul ^{32}P 33mer 5uM stock ✓
66ul x 14 = ~~924~~ 990ul H_2O ✓
90 x 14 = ~~1260~~ 1350ul mix A

	stop tubes			
Tfl/Vent in LTI SB ✓	x5	1-4	5-8	9-12
" in epicenter SB ✓		13-16	17-20	21-24
" in epicenter Tfl		25-28	29-32	33-36
vent*		37-40	41-44	45-48

1 no enz

2) each enzyme/buffer mix

mix A	90ul	90ul	90ul ✓
H_2O	8 ✓	6 ✓	4 ✓
undiluted enzyme (0.09 u/ul)	2	4	6

take 3, 6, 9, 12 min time points by removing 10ul of rxn to 5 ul stop in small tubes, keep on ice

start rxns 1 min apart

time on clock -				
	0	1	2	start
	3	4	5	stop
	6	7	8	stop
	9	10	11	stop
	12	13	14	stop

1 no enzyme control 50ul - 10ul Cheng, 2ul ^{32}P 33mer, 38ul H_2O, 50ul → 5ul + 2.5ul stop
45 A, 5 H_2O, remove 10 & stop

To Page No.______

Read & Understood by me, Polanco Date 6/9/95
Invented by [signature] 6-12-95 Date ~~6/7/95~~ 6/9/95
Recorded by [signature]

Project No._____

10 Book No._____ TITLE _____

From Page No.__

Note:
samples Vent / Tfl in LTI, 3 min 2 kV > only 5 ml of can was stopped - load mar
3 min 2 kV 4.5 inste

4:50 PM - ~6:15 PM 1700 V constant, ~45 W, gel was dried & put in P.I
* 39 & 46 may be underloaded due to problem expelling full vol. from

order: control, 1 → 48 where 1-12 are Tfl/Vent in LTI SB
of samples on gel 13-24 are Tfl/Vent in epicenter SB
25-36 are Tfl/Vent in epicenter Tfl
37-48 are Vent alone

T Pag N

Witn ss d & Und rsto d by m , D P Dolan

Dat 6/9/95

Invent d by 6-16-95

R cord d by Carolyn Combs

Date 6/9/95

Repeat unit assay for Tf1/V.
incorp of P.30 for stability of
Virus Tf1/Vent mixes of #'s J-
From Page No.
with 5 duplicate dilutions
Use 2 µl/of 1/250 dil for optimized signal/noise
and linearity vs (units)?

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Tf1 Chem unit assaying (same as P. 30) 47 →

Tf1 4.33 u/µl (P.T) 1/250 dil * 2 →

Tf1/Vent LTI SB 5-16-97 (Ning Guan's notebook) 1/250 2 →

Tf1/Vent Epicenter SB 5-16-97 1/250 dil 2 →

Tf1/Vent (Epicenter Tf1) 5-16-97 1/250 dil 2 →

Vf-50µl → 14°C, 10' → 10µl 0.5m EDTA → spot 40λ on

* all dilutions are done as 5 separate dilutions of 2µl Enzyme into 498 µl Tag dilution buffer

T Pag

Witness d & Understood by me, Dat 6/9/95 Inv nted by Dat 6-9-97
R c rded by

ge N .__

1	4163.00
2	4429.00
3	4636.00
4	4646.00
5	4349.00
6	4550.00
7	4529.00
8	4623.00
9	4350.00
10	4315.00
11	3995.00
12	4339.00
13	3732.00
14	4695.00
15	4428.00
16	3975.00
17	4584.00
18	4541.00
19	4297.00
20	4412.00
21	259.00
22	84613.00
23	87557.00

T Page No.____

sed & Und rstood by me,	Date 6/19/95	Inv nted by	Date 6-9-95
		Recorded by	

Test run of PEI plates - prior to turnover expt

No.

1/95

pose: To test how well a fresher batch of PEI plates can resolve dAMP from dATP and how tight/clean the spots are. This is being done prior to using this batch of plates for another TFI/Vent turnover experiment

ckground: Last time the dAMP spot did not resolve well from the yellow "junk" that runs near the 1M LiCl solvent front, making it difficult to cut out + count just the dAMP (without Pi) for accurate turnover results. We'll try washing a different batch of plates - from Jesse - in dH_2O + drying them 1st, before running samples and compare to unwashed plate. Also we see if running a whole plate gives better resolution than a ¼ plate

terials: PEI plates - from Jesse <- Machery Nagel Polygram cell 30 PEI/UV
Aldrich cat # 12,288-2
Kill soln = ~20mM dATP
20mM dADP
20mM dAMP
100mM CDTA
1M LiCl - Fresh, see recipe on next page

To Page No.____

d & Understood by me, | Date | Inv nted by [signature] 6/19/95 | Dat 6/12/95
Recorded by Carolyn Coombs

[redacted] Exhibit 107
Appl. No. 09/558,421 [redacted]

see P14 apparent exo present in PCR buffer
P44, no exo in Cheng buffer

Project No.______
Book No.______

TITLE Repeat apparent exo result for Tf1 on P14 with different primers ~~+ Mono + ...~~

From Page No.__

Exhibit L-107
Appl. No. 09/558,421

	①	②	③	④	⑤	⑥	⑦	⑧
10X PCR buffer	5 →						5	✓
50 mM MgCl2	10 ~~5~~µl →						15	✓
5X Cheng (no dNTPs) (see P20)				10µl →			10	✓
32P 33mer correct (P51) 5µM	2			2				✓
32P 23mer "AC" 5µM		2			2			✓
32P 42mer "fidel" 5µM			2			2		✓
see P12, 14 for method								
rTaq storage buffer							10	10
Tf1 4.33 u/µl (P.8)	*10 →							
H2O	31.5 →			28 →			31.5	28

$V_f = 50\mu l$

74°C. remove 10µl to 5µl cycle seq stop sol at 15 min, 60 min

run on 8% PAGE

* Zero time point:

1. mix buffer mix, 32P primer (MgCl2 if needed) and H2O. Volume =
2. remove 8µl to 2µl Taq storage buffer and 5µl cycle seq ... for 0 (no enzyme) time point.
3. now have 32µl of reaction mix left. preheat to 74°C, add 8µl Tf1 so Vf = 40µl again and remove 10 at 15 and 60 min to 5µl cycle seq stop sol.

To Page

Witnessed & Understood by me,	Date	Invented by	Date
Deanna Bolay (signature)	6/19/95	(signature) Recorded by	6-13-95

³²P oligos (follow P12, 14 for 5μM Cf.)

Project No.

Book N

ag N

	1	2	3	
5X Kinase buffer	4μl	→	→	✓
"arrest" 20μM 1389	5μl			✓
mer AC (P136,5) 100μM		1μl		✓
mer "fidel" 6-13-95 "old Temp" This is different from Fidel Temp P54 100μM			1μl	✓
ATP 10μCi/μl (ref 6-16-95)	2μl	→	→	✓
VK	1	→	→	
H2O	8	12	12	✓

← 23mer has 3' terminus A instead of G its called "AC".

← (5X less γATP than on P12)

Vf = 20μl

37°C, 30', 5', 55°C

22mer ~~fidel~~ fidel

51351 CKC (012)

56.89 nmoles primer
568.9 μl H2O

f = 100μM

To Page No.

sed & Understood by me,	Date	Invented by	Date
[signature]	6/19/95	[signature]	6-13-95
		Recorded by	

Incorporation and turnover by Vent alone
Time course on 0.1, 0.2 0.3 units Vent

Page No.

Purpose: To measure both incorporation and turnover by varying amounts of Vent over a 1 hr time course. These values will be compared to those of TFI/Vent mixes. Vent alone is a control for the stability assays of TFI/Vent mixes.

Background: NB 10 p. 41, turnover was not above background for Vent alone, when 0.09 units were used in a 20min time course. This time will try more enz. However, Roger had observed turnover over background when 0.15 units were used /100ul rxn. Turnover is expected to increase linearly w/ time even after incorporation has stopped, if Vent can bind at the nick. New PET plates may give better results than last time.

Materials: PET plates from Jesse, tested on NB 11, p. 15, Vent 2u/ul, Kill soln = NB 11 p. 14 → 20mM dATP, dTTP 100mM EDTA

activated DNA = gapped DNA made w/ DNase I

^{32}P α dATP - ref date 6/16/95

mix A: per 1, 100ul rxn:
- 63.786 ul H_2O
- 20ul 5x Cheng buffer
- 13.5ul activated DNA, 3.7 mg/mL, Cf = 0.5
- 0.5ul dATGC-TP, 10mM each, Cf = 50u
- 0.214 ul α ^{32}P dATP 10mCi/ml

make enough mix A for today + the next expt - TFI/Vent turnover
16x 98ul/rxn = 1568ul

(for 16 reactions) A
- 1020.6ul H_2O ~~qs~~ 1mL + 20.6ul ✓
- 320ul 5x Cheng ✓
- 216ul DNA ✓
- 8ul dATGC-TP - BRL Lot FB14001 ✓
- 3,424ul ^{32}P α dATP

7.5 x 10^7 ~~4.7 x 10~~ CPM/ul
~~294~~ 250/100 µl Reaction
7x10^6
VF 1569 µl use 98 µl/100 µl rxn

Vent dilutions - 2/4/95 Lot 17 spin + mix of Vent
LTISB = (1aq SB)
~~1st dilute 5x Cheng → 10ul 5x Cheng + 40ul H_2O-mix~~
① dilute Vent stock (2u/ul) to 0.15u/ul → 2ul Vent + 24.66ul ~~1x~~ LTISB Cheng
② dilute ① to 0.1u/ul → 10ul ① + 5ul ~~1x Cheng~~ LTISB
③ dilute ② to 0.05u/ul → 5ul ② + 5ul ~~1x Cheng~~ LTISB

T Pag No.

Read & Understood by me,	Date	Invented by	Date
Lucia Polanco	6/19/95	6-19-95	6/13/95
		Recorded by Carolyn Combs	

From Page No.

procedure: - deliver 10ul of kill soln to 1.5mL Eppendorfs ✻ 1-
- label tops 10' 20' 30' 40' 50' 60', set on ice until

rxn ✻	1	2	3	4
stop tube ✻	1-~~6~~	~~7~~-~~12~~	~~13~~-~~18~~	~~19~~-~~24~~

mix A 98ul/rxn — prewarm to 68°C →

0.15 u/ul Vent 2ul start rxns by adding enz., keep at 68°C in 9600

Lot# 17
opened 2-24-95

2ul ← Thin in ... TF1/Vent

0.10 u/ul Vent

0.05 u/ul Vent 2ul

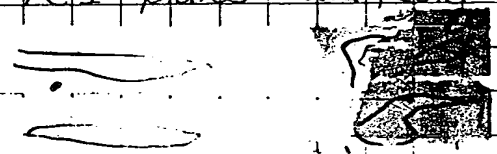
0.2 u/ul ... TF1/Vent ... in 0.1 ... Vent

no enz 2ul ~~tr Chenq~~
LTI $B
= Taq $B

100ul

- remove 10ul rxn to stop tubes at each time pt (10', 20', 40', 50',
- spot 10ul/GFC + 2ul PEI
L+ 10ul of mix A x 3 (for determination of specific activity)

Test of old (Baker) PEI plates - 2ul, cold kill soln spotted



- old plate → dAMP runs w/ solvent front junk

EC

- new plate → dAMP runs ~½ between solvent front & or

T Pag N

Witness d & Und rst od by me:	Date	Invent d by	Dat
Deanna Polump	6/19/95	6-15-95	6/13/95
		R cord d by Carolyn Combs	

Exhibit **109**
Appl. No. 09/558,421

Project No._____

Book No._____ TITLE Incorporation (pmoles)

From Page No.___

$\left(\frac{cpm}{\text{specific activity}}\right)\left(\frac{10ul\ rxn}{10ul\ spot}\right)\left(\frac{20ul}{10ul}\right)$

3 units	55	21752.00 - 936 pmoles
	56	40494.00 - 1742
	57	50701.00 - ~~2745~~ 2181
	58	63810.00 - ~~2723~~ 2745
	59	63423.00 - 2723
	60	61923.00 - 2663
1 units	61	12710.00 - 547
	62	21727.00 - 934
	63	32040.00 - 1378
	64	39939.00 - 1718
	65	43064.00 - 1852
	66	51401.00 - 2211
0.1 units	67	9060.00 - 390
	68	14810.00 - 637
	69	19948.00 - 858
	70	24421.00 - 1050
	71	31940.00 - 1374
	72	30490.00 - 1311
no enz.	73	420.00
	74	540.00
	75	299.00 $\bar{x} = 348$
	76	310.00 $n = 6$
	77	197.00
	78	323.00
	79	923,719.00
	80	973,931.00
	81	890,737.00

10ul spot of mix A for specific activity

$\bar{x} = 929,402$

The observed specific activity of mix A is 2x higher than anticipated by the following calculation:

$$\frac{10\ \frac{uCi}{ul}}{0.925^{2\ days\ to\ ref.\ date}} \times 2.2\times10^{6}\ \frac{cpm}{uCi} = 2.6\times10^{7}\ \frac{cpm}{ul} \times 3.4\ ul\ in\ mix\ A = \frac{8.7\times10^{7}\ cpm}{1568\ ul\ A} = 5.6\times10^{4}\ \frac{cpm}{ul}$$

$5.6\times10^{4}\ \frac{cpm}{ul\ A} \times 10\ ul\ A\ spotted = 5.6\times10^{5}\ cpm\ expected$

$9\times10^{6}\ cpm$

$9.3\times10^{5}\ cpm\ observed$

I don't know where the error came from, but the results should still be consistent within this experiment

To Pag No

Witnessed & Understood by me,	Dat	Invented by	Dat
Deneen Pokus	6/19/95	6-19-95	6/14/95
		Recorded by Paulina Pinsky	

Tfl/Vent in LTI SB: turnover and incorporation
5 replicates of 3 time points

ge N .

pose: To more accurately determine turnover by Tfl/Vent in LTI SB
- 5 replicates of the 5min and 10min +15min time points, ~~which~~ are within linear range of the assay. This data will used for the stability study.

ckground: > also see NB 10 page for a time course of turnover, 2 replicates ✓

aterials: new PEI plates,
mix A from 6/13/95
Tfl/Vent in LTI SB
Kill soln from NB 11 page 14 = 20mM each dA-mDTP-P
100mM EDTA

ocedure: - deliver 20ul Kill soln to 1.5ml stop tubes 1–18 ✓

rxn # s (good tubes)	1	2	3	4	5	6
top tube #	1-3	4-6	7-9	10-12	13-15	16-18

mix A 98ul → prewarm to 68°C

wick plate

l/Vent mix 2ul - add enzyme to start rxns
TI SB 2ul
2ul
2ul
2ul
2ul
~~2ul~~ ✓
~~no enzyme control~~
2ul LTI SB = Taq SB

incubate at 68°C
100ul

> At 5, 10, 15 min remove 20ul rxn to the 20ul Kill soln in stop tubes, mix well & keep on ice
> spot 10ul/GEC filter (1-15) + 12 = 27 spot rxn 6, no enz control 4x per time pt
2ul/PEI plate (1-15) 27 x 2 = 54 = 12 spots
16-1, -2, -3, -4
17-1, -2, -3, -4 18-1, -2, -3, -4 =

To Page No.

d & Understo d by me,	Dat	Invented by	Date
[signature]	6/14/95	6-15-95	6/14/95
		Recorded by Carolyn Coats	

[redacted]

22 *Results:*

Project No.
Book No.
TITLE

Turnover (pmol)
(cpm - background cpm / specific activity*)(40/20)(100/2)

SAM (odd = dAMP, even = dADP or dATP)	CPM1	Turnover (pmol)
1 > 5'	950.00	(950 - 495 / 419)(40/20)(100/2) = 109
2	72035.00	
3 >	1727.00	~~163~~ 294
4	71656.00	
5 > 15'	2488.00	476
6	67554.00	
7 > 5'	1176.00	163
8	72213.00	
9 > 10'	1768.00	304
10	69650.00	
11 > 15	2189.00	404
12	69544.00	
13 > 5'	1219.00	173
14	71519.00	
15 > 10'	1784.00	308
16	70384.00	
17 > 15	2314.00	434
18	69324.00	
19	1189.00	166
20	71061.00	
21	1914.00	339
22	72483.00	
23	2096.00	382
24	67757.00	
25	1032.00	128
26	70489.00	
27	1714.00	291
28	64939.00	
29	2289.00	428
30	64174.00	
31	612.00	
32	75976.00	
33	494.00	
34	79452.00	
35	421.00	
36	79068.00	
37	587.00	
38	78840.00	
39	460.00	
40	80087.00	
41	481.00	
42	78697.00	
43	419.00	
44	78893.00	
45	404.00	
46	76101.00	
47	450.00	
48	68672.00	
49	549.00	
50	67776.00	
51	498.00	
52	73381.00	
53	559.00	
54	72680.00	
55	620.00 spot near dAMP	
56	1714.00 spot near dADP/TP	

Side labels: samples 1–6 "TSI/vent replicate 1"; 7–12 "replicate 2"; 13–18 "replicate 3"; 19–24 "replicate 4"; 25–30 "replicate 5"; 31–38 "4 spots no enz"; 39–46 "4 spots no enz"; 47–54 "4 spots no enz"

$\bar{X}$ Turnover ± 1SD (n = 5)	% Turnover 100x (pmole dAMP/incorpora
5' ⇒ 148 ± 28	3.3%
10' ⇒ 307 ± 19	5.0%
15' ⇒ 425 ± 35	5.5%

background for turnover
$\bar{X}$ = 495 ± 69 cpm = 118 pmoles → samples are only 1 to 3 above background
n = 12

cc 6/14/95

T Pag N

Witnessed & Understood by me: Date 6/19/95
Invented by: 6-15-95 Date 6/14/95
Recorded by:

Page No.

Incorporation (pmol)

(cpm / specific activity)(100ul rxn / 10ul spot)(40ul / 20ul)

5' 93072.00 93072/419)(100/10)(40/20) = 4443
10' 107957.00 - 5153
15' 140583.00 - 6710

5' 107888.00 - 5150
10' 116159.00 - 5545
15' 157153.00 - 7501

5' 89224.00 - 4259
10' 129878.00 - 6199
15' 158185.00 - 7551

5' 86678.00 - 4137
10' 129770.00 - 6194
15' 146342.00 - 6985

5' 71757.00 - 3425
10' 127388.00 - 6081
15' 158825.00 - 7581

$\bar{X} \pm 1SD$ Incorporation

$n = 5$

5' ⇒ 4283 ± 620 pmol

10' ⇒ 5834 ± 467 pmol

15' ⇒ 7266 ± 395 pmol

no enz 5' 285.00
5' 355.00
no enz 10' 291.00
10' 300.00
no enz 15' 310.00
15' 262.00

background
$\bar{X}$ = 301 ± 31 cpm
≈ 14.4 pmoles
n = 4

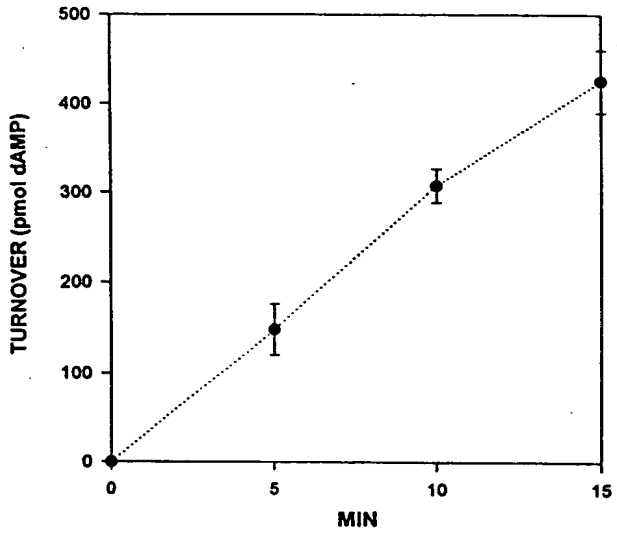
839570.00 10ul mix A
831885.00 spotted 3X
840299.00 $\bar{X}$ = 837,251

Specific activity = 837251 cpm × (100ul rxn / 10ul spot) / (5000 pmol) 4 = 419 cpm / pmole (nt)

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
Susan Polanyi	6/19/95	Recorded by Carolyn Combs	6/14/95

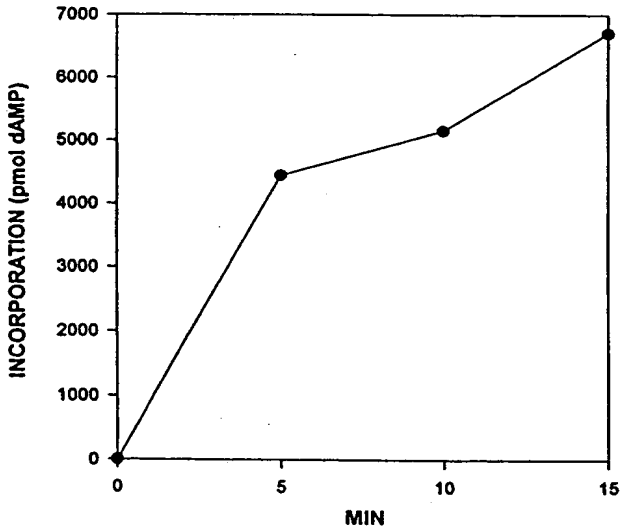
PROOFREADING: Tfl/VENT



6/15/9
ee

- Background was 118 pmd, so the best signal to noise occurs at 15 min 13.6x backgrou However, by 15 min, incorporation is slowing down as gaps are filled in. At 15 min some turnov is occuring at nicks - not a good model of a PCR reaction
  * Trade off between good model and s
- Linearity of the time points is good
- A 10% drop in activity would be detected by this assay, using 5 replicates.

POLYMERIZATION: Tfl/VENT



- Incorporation falls off after 5 min, because gaps are filled by high Tfl polymerize activity. After that, turnover occurs at nicks.
- % turnover increases beca DNA synthesis is slowing d while turnover keeps com at the same rate.

Witnessed & Understo d by me,	Date	Inv nt d by	Date
Dorcas Pokey	6/26/95	R corded by Pawlyn Combs	6/25/95

...ag N ___

...ults:

...lig Turnover

% Turnover: (pmoles dAMP / pmoles incorporation + dATP) x 100

= dADP + dATP
= dAMP

Turnover (pmoles) dAMP = $\left(\frac{\text{cpm} - \text{background cpm}}{\text{specific activity}}\right)\left(\frac{20}{10}\right)\left(\frac{100}{2}\right)$

ex. $\left(\frac{1776-807}{465}\right)\left(\frac{20}{10}\right)\left(\frac{100}{2}\right) = 208$

ex. $\frac{208}{208+936} \times 100 = 18.2$

	CPM1	Turnover (pmoles) dAMP	% Turnover
>10'	1776.00 86470.00	208	18.2
>20'	2994.00 86141.00	470	21.2
>30'	4209.00 91512.00	732	25.1
>40'	4983.00 85588.00	898	24.7
>50'	6822.00 94359.00	1290	32.1
>60'	7013.00 85869.00	1330	33.3
>10'	1216.00 79679.00	88.0	13.9
>20'	2179.00 83426.00	295	24.0
>30'	2954.00 81631.00	462	25.0
>40'	3716.00 83944.00	623	26.6
>50'	4469.00 85258.00	788	29.8
>60'	5283.00 87259.00	963	30.3
>10'	1223.00 85430.00	89.5	18.7
>20'	1807.00 90067.00	215	25.2
>30'	2316.00 88894.00	325	27.5
>40'	2953.00 84914.00	462	30.6
>50'	3572.00 90268.00	595	30.2
>60'	3815.00 92711.00	647	33.0
>10'	792.00 81173.00		
>20'	749.00 87079.00		
>30'	655.00 87371.00		
>40'	880.00 86383.00		
>50'	785.00 86929.00		

$\bar{x}$ dAMP = 807 cpm is background = 174 pmoles

specific activity =

$$\frac{929{,}462\ \text{cpm} \times \frac{100\,\mu l}{10\,\mu l}}{(5000\ \text{pmole})(4)} = 464.7\ \frac{\text{cpm}}{\text{pmole nt DNA}}$$

CPM1

>60' 978.00
90674.00

To Page No.______

Witnessed & Understood by me	Date	Invented by	Date
[signature] Polanco	6/19/95	[signature] 6-19-95	6/19/95
		Recorded by [signature]	

From Page No. Results: Relative mobility, RM = distance from origen to cente

6/20/95 CSC

	SAM	CPM1	distance (cm) from origen to center of rectangle	↓ RM
				.45/9 = 0.072
no enzyme, front at 9cm	1	21456.00	0.65	0.17
	2	436.00	~~0.85~~ 1.5	0.21
	3	217.00	~~1.65~~ 1.9	0.26
	4	128.00	2.3	0.30
	5	105.00	2.7	0.41
	6	232.00	3.65	0.54
	7	73.00	4.9	0.66
	8	68.00	5.9	0.77
	9	78.00	6.9	0.91
	10	88.00	8.2	
60' vent, front at 9cm	11	18209.00	same data as above	
	12	572.00		
	13	153.00		
	14	87.00		
	15	175.00		
	16	1759.00		
	17	119.00		
	18	78.00		
	19	151.00		
	20	160.00		
90' vent, front at 9cm	21	17132.00	same data as above	
	22	42.00		
	23	46.00		
	24	99.00		
	25	314.00		
	26	2236.00		
	27	60.00		
	28	72.00		
	29	98.00		
	30	174.00		
no enzyme, front at 13cm	31	21668.00	1	0.077
	32	250.00	2.25	0.17
	33	175.00	2.75	0.21
	34	112.00	3.25	0.25
	35	115.00	3.75	0.29
	36	201.00	5	0.38
	37	63.00	6.5	0.5
	38	71.00	8	0.62
	39	101.00	10	0.77
	40	100.00	12	0.92
60' vent, front at 13cm	41	17710.00	same	
	42	326.00		
	43	133.00		
	44	125.00		
	45	104.00		
	46	1786.00		
	47	59.00		
	48	56.00		
	49	155.00		
	50	121.00		
no enzyme, front at 18cm	51	16715.00	same	
	52	400.00		
	53	225.00		
	54	82.00		
	55	91.00		
	56	2316.00		
	57	76.00		
	58	60.00		
	59	100.00		
	60	194.00		
90' vent, front at 18cm	61	21188.00	1.25	0.
	62	499.00	2.75	0.
	63	195.00	3.25	0.
	64	86.00	3.75	0.
	65	83.00	4.25	0.
	66	99.00	4.75	0.
	67	81.00	5.25	0.
	68	75.00	5.75	0
	69	139.00	7.25	0.
	70	73.00	9.25	0.
	71	66.00	11	0.
	72	53.00	13	0.
	73	130.00	~~14~~ 16	
60' vent, front at 18cm	74	19038.00	same	
	75	333.00		
	76	136.00		
	77	79.00		
	78	59.00		
	79	90.00		
	80	59.00		
	81	73.00		
	82	1811.00		
	83	70.00		
	84	50.00		
	85	55.00		
	86	262.00		
90' vent, front at 18cm	87	18132.00	same	
	88	449.00		
	89	249.00		
	90	72.00		
	91	56.00		
	92	76.00		
	93	70.00		
	94	66.00		
	95	2231.00		
	96	99.00		
	97	45.00		
	98	68.00		
	99	234.00		

T Page

Witness d & Underst d by me,	Dat	Inv nted by	Dat
Deserie Polanco	6/26/95	R c rd d by	6/20/95

Primer degradation by TFI/Vent-Epicenter + Vent alone Pr j ct No.____ B k No.____
Time course + 5 replicates on 12% gel

e N .__

-pose: To measure 3'→5' exonuclease activity of TFI/Vent mix using the primer degradation assay. The data will be part of the stability study on the mix. The following changes to the assay will be made in hopes of obtaining more accurate data on the rate of primer degradation:
i) 12% gel will be used instead of an 8% gel - may give better peak resolution. Last time, peak shoulders and double peaks were a problem during quantitation
2) 44mer Fidel will be used instead of 33mer - correct - the 33mer could form primer dimers &/or a hairpin that may have altered the degradation rate.
3) By doing 5 replicates, we can access the accuracy of this assay as compared to the turnover assay.
Primer degradation by Vent alone will also be measured.

kground: Note - although we tested TFI/Vent mixes on p. 9 NB 11, only the TFI purchased from Epicenter is TFI. LTI's TFI is really Tth. That is why we are not doing any stability tests on LTI's enzyme, until a new TFI alone is obtained.
- the amount of enzyme and time course of this experiment are known to be in linear range of assay from the ealier expt on p. 9 NB 11.

erials: γ^{32}P-end labeled 44-mer Fidel
fresh mix A
12% denaturing sequencing gel
stop soln
TFI (Vent) mix made w TFI purchased from Epicenter SB, Vent 10 of #17

or recipe for 12% gel, 100mL: 48g urea
30mL, 40% acrylamide:bis mix
10mL, 10x TBE
dissolve by stirring + low heat
+ 600µl 10% AP (100ug/µL)
q.s. to 100mL w/ H_2O - squirt bottle
30µl TEMED
100mL

To Page No.____

& Understood by me,	Date	Invented by	Date
Clamp	6/26/95		6/21/95
		Recorded by Carolyn Combs	

Project No._____
Book No._____ TITLE _____

No.__

A: 90 ul per rxns, enough for 15/9 rxns

15
6ul x 9 = 594 ul H_2O 990ul
20ul x 9 = 150ul 5x Cheng buffer 300ul
4ul x 9 = 36 ul ^{32}P-44mer Fidel, 5uM stock C.f=200n
90 x 9/15 = 540ul 1350

edure:

label the 44-mer Fidel primer - 70.3ul H_2O
24ul 5X Kinase buffer
6 ul 44mer Fidel, 100uM st
13.7ul $^{32}P\gamma$ ATP, 10uCi/ul
ref date = 6.
6 ul PNK 1u/ul
120ul

37°C, 30min
55°C, 5min
store at -20°C overnight

deliver 5ul Stop soln to 9600 tubes * ~~1-12~~ 1-28 / label rxn tubes 1-
make mix A

rxn #	1	2	3	4	5	6	7	8	9	10	11	12
H_2O	8	8	8	8	8	8	8	8				
mix A	90	90	90	90	90	90	90	90				

preheat to 65°C in 96CC, start by adding e

Ep: 5B * / no enzyme 3ul — 2ul TFI / Vent - Epicenter — 2ul Vent

At each time pt remove 10ul rxn to the 5ul stop soln
- heat 90°C
- spin

no enzyme 0', 10' replicas: 4' 6' at TFI / Vent
time course 2', 4', 6', 8', 10', 20' w/ TFI / Vent

To Page N

& Understood by me,	Dat	Invented by	Date
	6/26/95	Record d by Dawlen Pomb	4/21/95

g N. _____

icenter SB for no enz control: final 100mM NaCl, add solid to LTI SB
nd to dilute vent with. final 50mM Tris
now at 20mM

5mL Taq SB = LTI SB ✓
91 ul 1M Tris 7.5 - premade by LTI ✓
62.5 ul 1M Tris-HCl ✓ → 157.649/mole
153.5 ul glycerol ✓
0.031 g NaCl (58.449/m) ✓

$\frac{1\text{ mole}}{L} \times 10\times10^{-3} L = 0.01$ mole

$\frac{g}{157.649/m} = 0.01$ mole

= 1.5764 g Tris·HCl LTI
+ 10 ml dH_2O

Vent dilution in Epicenter storage buffer = 22.2x dilution to 0.09 u/ul
2 ul Vent stock (2 u/ul) w/ p2 pipetman → 1st spin down + vortex Vent stock
+ 42.4 ul Epicenter storage w/ p200 pipetman
44.4 ul vortex to mix

mples were heated to 90°C, 5' in 9600 prior to loading.

sample #'s: 1 = no enz 0 min
2 = no enz 10 min
3 = TFI/Vent 2' min - not preheated = may be off
4 = " 4'
5 " 6'
6 " 8'
7 " 10'
8 " 20'
9 (1) TFI/Vent 4' → 1
10 " 6'
11 (2) 4' → 2
12 6'
13 4' → 3
14 6'
15 4' → 4
16 6'
17 4' → 5
18 6'
19 Vent 4' → 1
20 6'

21 4' → 2
22 6'
23 4' → 3
24 6'
25 4' → 4
26 6'
27 4' → 5
28 6'

2nd load 1,3-28,12 2nd run longer
2, 3 - 28, 8
1st load 1,2, 4-28,2 1st
1,19-28,2 no times course

To Page No. _____

d & Understood by me,	Date	Invented by	Date
Solamp	6/26/95		6/21/95
		Recorded by Carolyn Combs	

Project No._____

Book No._____ TITLE _____

om Page No.____ sample #

1st loading: 1, 9-28, 2 (no time course) ~3:15 pm, rate 0.7cm / 8 min ≈ 0.1 cm/min

2nd loading 2, 3-28 w/ time course ~5:15 pm, run until BØB reaches bottom. At this time ~10 bases will have run. This will be our whole gel loading to see the whole ge most of the products

Gel was run at 1700V constants for a total of 5.5 hrs.

To Page N

n ssed & Understood by me,

Date 6/26/95

Invented by

Dat 6/21/95

Recorded by

Exhibit 114
Appl. No. 09/558,421

Project . Book No. TITLE M13 PCR system: Taq and Tne

From Page No.

(40 Rxns)

PCR mix:

LTI 10x PCR buffer	400 µl
50 mM $MgCl_2$	120 µl (1.5
4 dNTPs, 10 mM each	80 µl (200
M13 RF 1 pg/µl ▲ see page 42 for dilution	40 µl (1p
M13-6301 (anchor), 20 µM	80 ← (400 primer
H_2O	3160
	3880 µl

(18 Rxns)

(in Falcon tube)

	1	2
	1746 µl	1746 µl (5 un 100 µ
~~H_2O~~		~~15.5 µl~~
rTaq 5 u/µl	18	
Tne ~~36 u/µl~~ 5 u/µl		~~2.5 µl~~ 18 µl
	1764 µl	1764

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
[1]	98 →															
[2]									98 →							
µl 20 µM primer see page 42-43																
M13 6621	2								2							
7069		2								2						
407			2								2					
806				2								2				
1491					2								2			
2506						2								2		
3972							2								2	
5464								2								2

100 µl

make 2 sets of
1-16 gets elongation ...
1'-16' gets elongation ...

RF
1 pg M13/100 µl
= 3 x 10^{-15} mole nt
= 2 x 10^{-19} ~~[illegible]~~ mo...
(2x10^{-19})(6.0 x 10^{23} molecules/...
= 125,000 molecules circles

94°C, 1 min → 30 cycles: 94°C 15 sec, 53°C 30 sec, elongation is 6 min for PCR 1-16 and 2 min for 1'-16' → cont. on

To Page

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/26/95	[signature] Recorded by	6-23-95

T-RC- and Tag with Cheng buffer
in gap DHFR

N .

	1	2	3	4
5 x Cheng buffer (w/o dNTPs) (w/o P20, 10)	10	10	10	10
10 mM dNTPs	1	1	1	1
Human spleen genomic DNA 200 ng/µl	1.25	→	→	→
2112 10 µM	1	—	1	—
2113 10 µM	1	—	1	—
5 u/µl Tag	0.5	0.5		
rxn 5 u/µl H2O	35.25	33.25	0.5 35.25	0.5 33.25
	50 µl			

T Page No.

d & Understood by m ,	Dat	Inv nt d by	Dat
B. Cheng	6/26/15	R cord d by	6-23-15

g No.__

SigmaPlot regression lines:

1) for Proofreading: Tfl/Vent the slope = 28.676 pmole/min, $r^2 = 0.978$

2) for Polymerization: Tfl/Vent the slope = 417 $\frac{pmole}{min}$, $r^2 = 0.873$

Units of Tfl/Vent proofreading: $28.68 \frac{pmole}{min} \times \frac{30 min}{10,000 pmole/2ul} = 0.043 \frac{u}{ul}$

Units of Tfl/Vent polymerization: $417 \frac{pmole}{min} \times \frac{30 min}{10,000/2} = 0.63 u/ul$

expect: 0.045 u/ul

Conclusions

1) The turnover assay can detect a 10% loss of 3' exo activity in Tfl/Vent mixes. By repeating the assay more frequently and/or with more replicates the error may decrease so that a 5% loss of activity could be detected.

2) Early time points, before 10min, reflect turnover during DNA synthesis - the best model of PCR. Later time points reflect turnover during DNA synthesis plus turnover at DNA nicks - not such a good model of PCR. However, the later time points give better data because the signal to noise ratio is higher (3.6x versus ~1.3x early on). Both. All 3 time points should probably be done during the stability study.

3) Turnover by Tfl/Vent mix is about 3x higher than by Vent alone. This result was observed in an earlier experiment too (pp10, 10): Tfl may create more mismatches for Vent to turnover than when no Tfl is present.

↑ not true: exo in Tfl/vent is only ~ 2x higher than for vent alone (as mistake on P 40, 10). In this experiment (pp 7, 11) the vent alone of 2 pl of 0.1 u/pl is the one we should compare to the Tfl/Vent mix.

To Page No.____

I & Understood by me,	Date	Invented by	Date
[signature]	6/26/95		6/24/95
		Record d by Carolyn Combs	

No.

PLATES

Both the dATP+dADP peak and dAMP peak become more spread out on the PEI plates as the solvent front runs further.
The distance between the two peaks becomes greater as the solvent front runs further: ~0.5cm vs 2cm

Conclusion: For best resolution of dAMP from dATP+dADP on PEI plates, run the LiCl solvent front to the top of the plate, ~16 cm from origen.

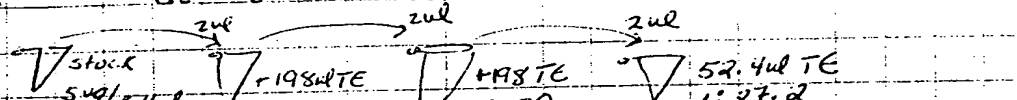
To Page No.

d & Understood by m ,	Date	Inv nted by	Date
Paul Polaney	6/26/95	Recorded by Carolyn Combs	6/24/95

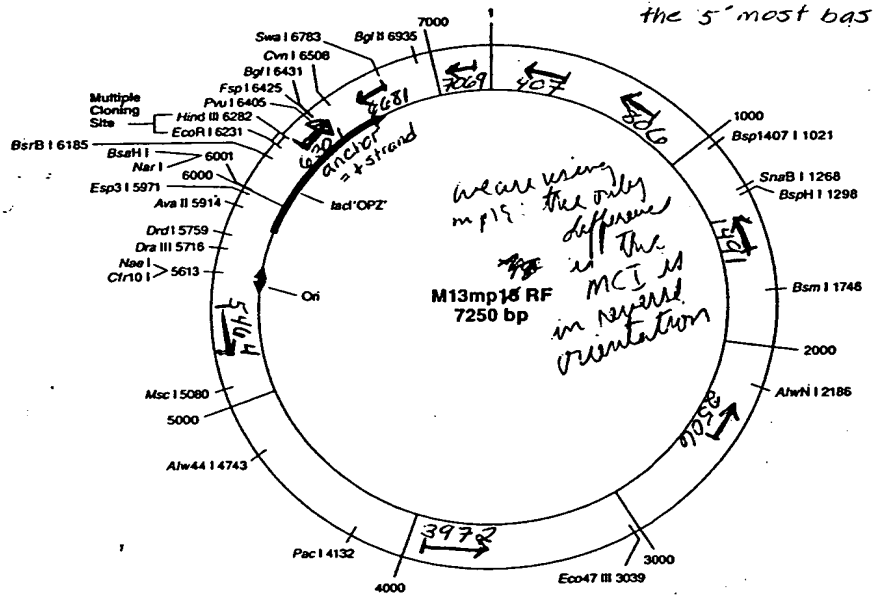
Project No._____
Book No._____ TITLE _____

rom Page No.__

Dilution of M13 RF to 1 pg/ul in TE: stock lot CN4132, 5ug/18.4ul
do serial dilutions to 1/2.72x10^5



6/20/95



M13mp19 primer
→ 20mers
→ 9 G+C
→ 11 A+T
→ Tm = ~58°C

primer name	PCR product length (bases)	sequence
M13-630i anchor (=+strand)		5' GTTTTACAAC G TCGTG
M13-6681 (=-strand)	380	5' TTCC TGTAGCCAGC TTTC
M13-7069 "	768	5' ATGCCTGAGTAATGTGTAGG
M13-407 "	1356	5' GAAGCAAAGCGGATTGCA
M13-806 "	1755	5' TTA TACCAG TCAGGACGT
M13-1491 "	2440	5' AGCTTGATACCGATAGTTGC
M13-2506 "	3455	5' CGACAGAATCAAGTTTGCC
M13-3972 "	4921	5' AATCG CAAGACAAAGAACG
M13-5464 "	6413	5' GTA TAACGTGCTTTCCTC

T Pag N

Witnessed & Understood by me,	Dat	Invented by	Date
	6/26/95	Record d by Pandora Pombo	6/24/95

e No.__

paration of primer stocks and dilutions:
Primers were made by Gibco BRL Custom Primers order# 510790A
Each ~~d~~ primer was ~~dilut~~ resuspended in sterile dH_2O at a Cf = 100uM
-spun down, H_2O added, 2min RT, vortex, invert

primer	nmoles/tube	volume of dH_2O added to make Cf = 100uM
3-6301 anchor	54.7	547 ul
3-6681	44.09	440.9
3-7069	~~39.14~~ 57.29	~~391.4~~ 572.9
3-407	39.14	391.4
3-806	51.92	519.2
3-1491	69.49	694.9
13-2506	66.34	663.4
13-3972	34.68	346.8
13-5464	42.45	424.5

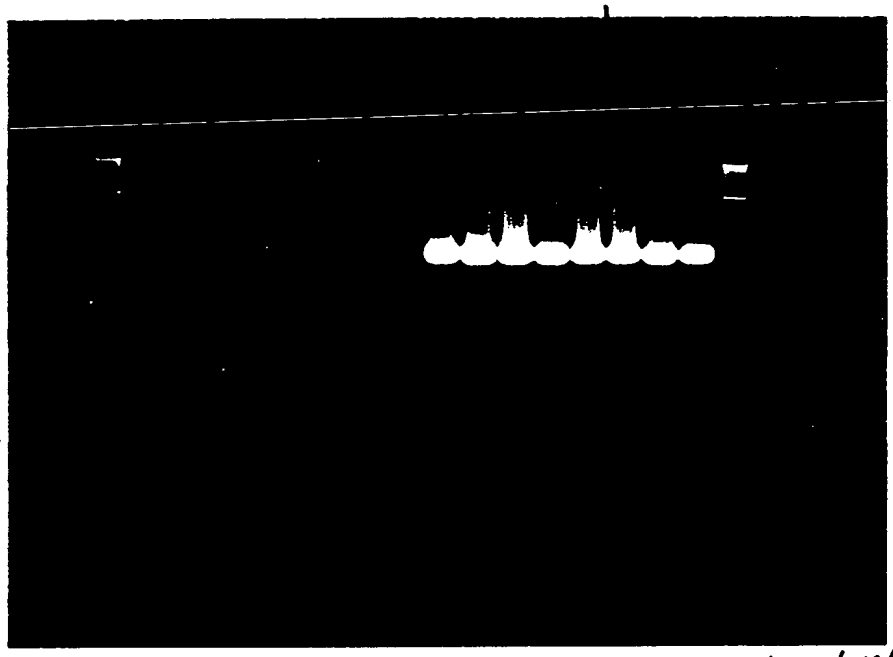
~~Each~~ 20uM aliquots of each primer were made from the 100uM stocks:
⇒ 1:5 dilution, 40ul of 100uM primer stock
+160ul dH_2O-sterile
200ul for each primer except m13-6301 anchor
1:5 dilution of m13-6301 anchor, 200ul of 100uM stock
+800ul dH_2O-sterile
1ml

uation of PCR expt from p. 40
2ul of 1Kb ladder and 10-18ul of PCR products were run on a 1% TAE
garose gel at 190V (~180mA, 40W)
o gel recipe: 220ml 1x TAE buffer: 2L 1x TAE
2.2g agarose w/ 70ul EtBr at each end
wt = 435.6g, boil, reweigh & add H_2O back to orig. wt
stir & cool
add 15ul, 10 mg/ml EtBr & pour into rig w/ quarters

To Page No.____

d & Und rst od by m ,	Date	Inv nted by	Dat
olemp	6/26/95	R corded by Carolyn Combs	6/26/95

From Page N .__



taped into book 6/26/95 cc

> Since most of the rTa PCR runs contained suc low level of product, tried loading more ul in or to be able to visualize products better

> No specific products seen in the Tne lan

taped into book 6/26/95 cc

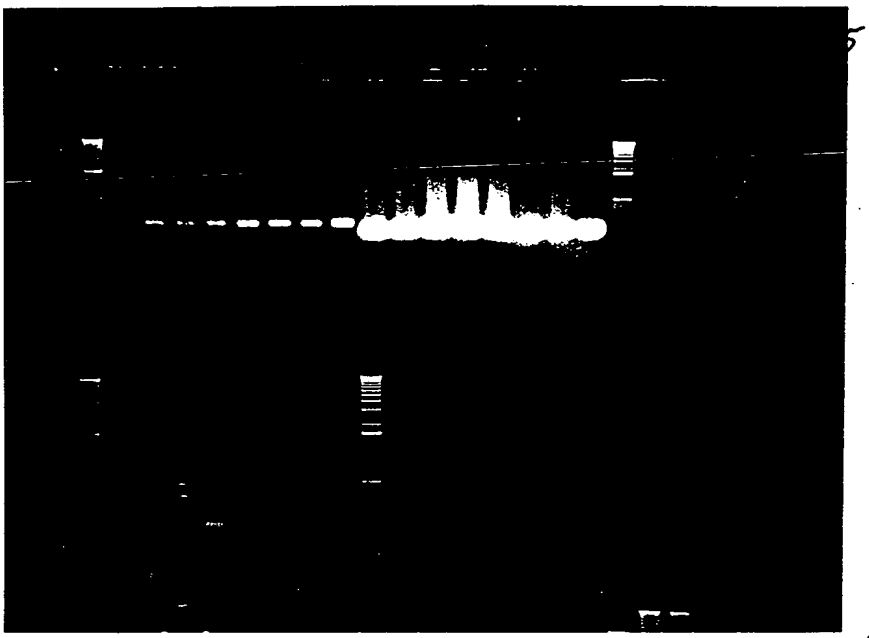
> note false product 1491 primer
- only 1 band of 2 was expected
- instead there are ~0.5-1Kb and 2-3

> Note false product m13 2506 primer - should be ~3.5Kb
> also a short, false product w/ m13-5464

To Pag

Witn ssed & Und rsto d by me,	Dat	Invent d by	Dat
	6/30/95		6/26/95
		Rec rd d by Carolyn Lamb	

g N .__



> 6' extention time did not give more product. - worse yield than with 2' extension

cc taped into book 6/26/95

To Page No.____

d & Understood by me,	Dat	Inv nt d by	Date
SD Kemp	6/30/95	Recorded by Carolyn Combs	6/26/95

113 PCR system: optimizing annealing temperature

[A] (2 Rxns)

LTI 10x PCR buffer	120 ✓
50 mM $MgCl_2$	36 ✓
4 dNTPs, 10 mM each	24 ✓
M13 RF 1 pg/µl (lot# CN4132)	12 ✓
M13 6301 "anchor" 20 µM	24 ✓
rTaq 5 u/µl	12 ✓
H_2O	947 ✓
	1.176 ml

25 rxns

51, 49, 47°C annealing temp w/ 1st 3 oligos program 19, 94°C
18, 94, 15"
- 51, 3"
70, 2"

	1 2 3	4 5 6	7 8 9
[A] 97µl →			
6681	2	2	2
7065	2	2	2
405	2	2	2

Rxn = 100 µl

14 ~~15~~	9600	51°C annealing temp	1-3
15		49°C	4-6
16		47°C	7-9

1 — 9 →

10ul 10Kb ladder

10ul 10Kb ladder

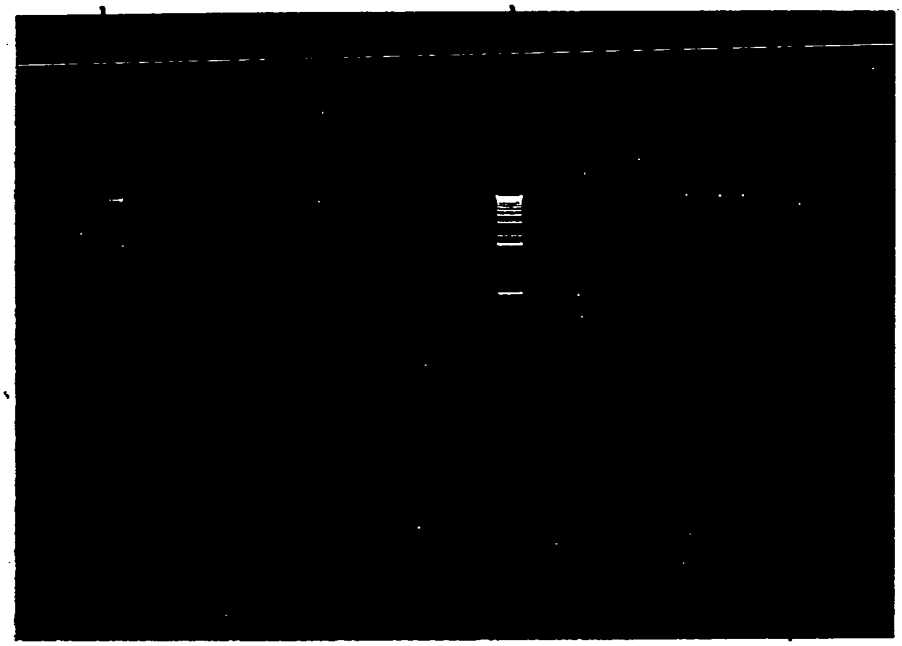
% gel: ~~2.64g agarose + 220ml~~ / ~~220ml~~

3.36 g agarose + 280ml TAE wt = 480.3g

To Page No. _____

Read & Understood by me,	Date	Invented by	Date
[redacted]	6/30/94		6/24/95
		Recorded by [redacted]	

Result:



6/20/95 cc

primer combo	expected product length (bp)	observed product length (bp)
anchor + 6681	380	✓ looks like 380 relative
anchor + 7069	768	✓ 768 "
anchor + 8407	1356	✓ 1356

> specific products of the right length were made, but the yield was still v. low. Eve thought lowering the annealing temp might result in a higher yield. It did not. The lanes on the right side of the gel just look a bit darker because the light box is brighter on that side (note how the 10Kb ladder looks more intense on right side even though load was loaded on left & right sides. Also note

> Next will try to increase the yield by using denaturation time (from 15" to 30" - Neil's suggestion), [Taq], # cycles, [primer] - in case the anchor primer has a hard time annealing due to 2° struc. If it does, than lowering annealing temp would exacerbate the problem.

Witness d & Underst d by m ,	Dat	Inv nt d by	Dat
[signature]	6/30/95		6/26/95
		R c rded by [signature]	

Δ denaturation temp, Δ[Taq]
Δ cycle number, Δ [primer]

e N .—

	[A]	10 Rxns
TT 10x PCR buffer	100 ✓	
50 mM $MgCl_2$	30 ✓	
4 dNTPs 10 mM	30 ✓	
413 RT (Lot FAS70) 1 pg/μl opened 6-26-95	10 ✓ →	different lot than on p. 40
H_2O	740 ✓	dilution of stock
	900 μl	stock 370 ug/ml → 2ul → 198ul H_2O → 2ul → 198ul H_2O → 10ul → 360ul H_2O
		1/100, 1/100, 1/37
		= 3.7×10^5 fold dilution

#	1	2	3	4	5	6	7	8
	90 μl							
	5	1	4		5	1	4	
30 Anchor (413-40 Anchor)	48	47			47	48		✓

1:1 mix of anchor + 407
30 ul 20 uM stock anchor
30 ul 20 uM 407
60 ul, 10 uM each
(400 or 800 nM oligo each)

5 ng/μl	1	1	2	2	1	1	2	2 ✓

94°C, 1' initial denaturation

15" denaturation 94°C
↓
55°C, 30 sec
↓
70°C 2 min
Lab 15, 9600

30 sec denaturation 94°C
"
"
Lab 16, 9600

30 and 40 cycles
↳ 20ul rxn 1-8
+ 2.3 10x LD
Freeze ON

3:15 pm
↳ left in PCR machine, at 4°C ON

To Page No.

& Understood by me,	Date	Invented by	Dat
	6/30/95	Recorded by Carolyn Coats	6-26-95

50

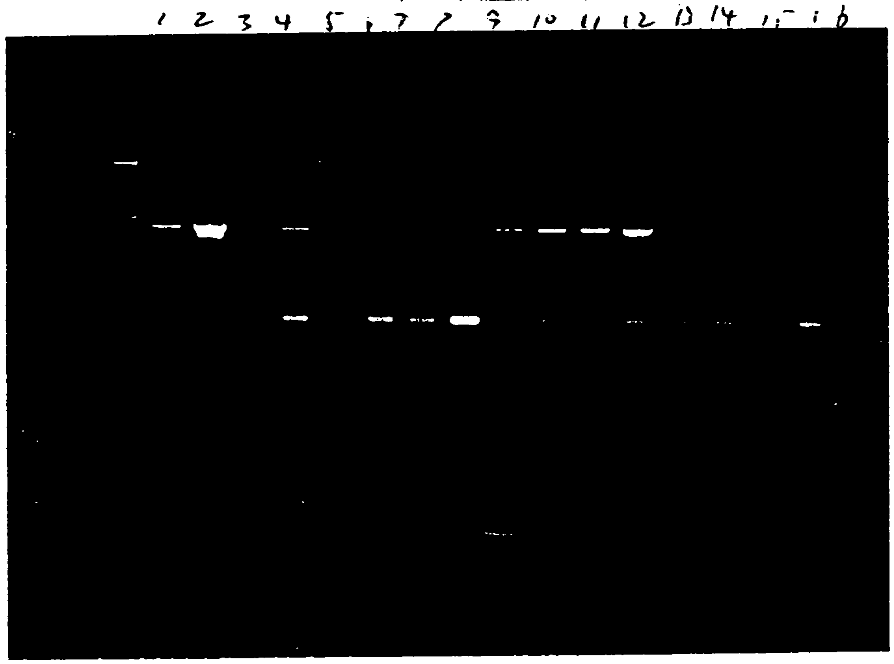
Project No.______ Results P. 49

Book No.______ TITLE

From Pag No.__

denaturation time	15 sec								30 sec							
units Taq/100λ	5				10				5				10			
primer (nM)	400		800		400		800		400		800		400		800	
cycles	30	38	30	38	30	38	30	38	30	38	30	38	30	38	30	38

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



← 1356

← primer dimer

0. tube 1 compared to tube 3 on P48 shows new M13RF only gives little or no improvement to yield at 30 cycles

1. lower [primer] (400 nM) is best (tubes 1, 2 vs 3, 4) (when denaturation is for 30 sec, 400 vs 800 nM primer about equal)

2. Lower Taq is best: only primer dimer made for 10 (#5-8 and 13-16)

3. 38 cycles made more product than 35 cycles (eg tube 2 vs 1)

4. 30 sec denaturation gave less product than 15 sec.

To Pag No.

Witnessed & Understood by me, [signature] Date 6/30/95

Invented by [signature] Date 6-26-95

Recorded by [signature]

g No.

Therefore:

keep 15 sec denaturation

Try even less primer (eg 100 200 300 400 nM)

Try even less Tag (2, 3 4, 5 units)

Try diffent apta number (30 – 40)

Try M13 6301 (another) alone and with other primers with no target to look for primer dimer

Try more M13 RF target
eg. 0.1 pmol – 10 n mol

try R1 (or Bam, HindIII etc) check buffer salt cut
+
PCR with no purification

Test all primers with test conditions

To Pag No.

ed & Understood by me,
50 lays

Date
6/30/09

Invented by

Recorded by Carolyn Conby

Date
6-26-09

TITLE 1.1X [illegible] stability assays 4°C storage (see P121,9) −20° and −70°C. freeze Thaw

From Pag No.

	Rxn #	assay ul	
# 10 (P121,9): no det	1-3	2	✓
# 11 " 1.1X	4-6	3.64	✓
Tanp #11 : 1.1X	7-9	3.64	✓
Tag 125 dil (same as P121,9)	10-14	2	✓
1.1X May 8 1995	15-17	2	✓
1.1X field test	18-20	2	✓
1-27-95 −20°C	21-23	2	✓
−20°C 5/24/95	24-26	2	✓
−70°C 5/24/95	27-29	2	✓
*10 freeze thaw	30 32	2	✓
20 freeze thaw	33 35	2	✓
30 freeze Thaw	36 38	2	✓
Fr 13-17 5200 Thl			
P 25,10			
1/100	39	2	✓
1/800	40	2	✓
1/200	41	2	✓
Thr 5-7-95 (~70.7 u/ul on P25)			
1/8000	42	2	✓
	43	2	✓
	44	2	✓

This is 5 month point for 4°C studies (same as P121,9; 154,9; 174,9; 37,10 = 0, 1, 2, 4 m (154,9 is 0 time point, P 38,10 is 1 month)

called "new" on P 34, 10
called "old" P 34

Joe S. took aliquot from samples o on 1-27-95 save freeze thaw stored at −20°C, 0° 5 months at with unknown effect from freeze/thaws

from 1.1X May 8 1995 (compare to Rxn # 15-17 above) 1 month at −20°C with no extra freeze th
from 1.1X May 8, 1995 – 1 month at −

* used 1.1X May 8, 95 (above) "new" in drying EtOH → 30°C bath re start with 60 µl and take out at 10, 20, 30 freeze thaw

repeat of P25 for Thr did 5200 fractions look activity at 4°C

47 µl Tag unit assay mix (P120,9) in each 20 µl
10, 74°C
Kill with 10 µl 0.5 M EDTA, spot 4 µl on G-FC

To Pag

Witnessed & Understood by me, Dat 6/30/95
Inv nt d by Dat 6-28-95
R c rd d by

Results P.J 2 Appl. No. 09/558,421 **Project N** ______ **Book N** ______ 5:

No.		a	u/l relative to Tag (#10-14)		percent of zero time for P122/9
1	1597.00				
2	1752.00				
3	1760.00				
4	7709.00	6760 ave	.030		94%
5	6150.00				
6	6422.00				
7	4510.00		.025		72%
8	5085.00				
9	5662.00				
10	8620.00	8931 CPM ave	.04 (by definition)		
11	9351.00				
12	8531.00				
13	8321.00				
14	9832.00				
15	5618.00	5632 ave 5632/8931 ·.04	.025	.025/.023	109%
16	5895.00				
17	5384.00				
18	5128.00	5215 ave 5215/8931 (.04) =	.023 u/l	.023/.023 ≠ 100%	
19	5036.00				
20	5481.00			↑ p34.97 for start	
21	3989.00	3673 ave it's been at -20°C for ~5 months + 4 freeze thaws; it came from tube #11 above (tubes # 4-6)		from .054/μl measured above (#4-6)	54% recovery
22	4058.00				
23	2971.00				
24	5931.00	5921 → conclude -20°C loses activity			
25	5591.00	1 month -20°C from 5-8-95 1.1X (#15-17 above)			
26	6242.00				
27	5891.00	5712 sample from 1.1X of 5-8-95 (see tubes 15-17 above) ⇒		no activity lost for 1 month at -70°C	101%
28	5381.00				
29	5865.00				
30	5644.00				
31	5407.00	5440	started with 1.1X (stored only at 4°C) -7.45 (see #15-17 above)	conclude no loss of units for even 30 Freeze Thaws	97%
32	5271.00				
33	5362.00	5405			96%
34	5494.00				
35	5361.00				
36	5556.00	5138			100%
37	6159.00				
38	5200.00				
39	138.00	—	TNE died at 4°C of 5200 col of 5-18-95 got 7.7 u/l on P.25		
40	287.00				
41	137.00				
42	1014.00	ave - BKGD = 627 cpm = 27.3 pmol	32.8 u/μl	agrees with 36 u/l	
43	960.00	Lg Flymgot and I got P.13, My 70.7 u/l			
44	1092.00	of P25 may be wrong; TNE is stable		will conclude Li's until more data is available	
45	395.00	BKGD			
46	110131.00	2X mix	68.9 CPM/pmol		
47	110429.00	2X mix			

To Page No.____

Read & Understood by me,	Date	Invented by	Date
[signature]	6/30/95	[signature] Recorded by	6-30-95 6-29-95

May 8, 95

3 batch:

① 17 sample
original buff. formulation
created
4°C RT -20°C Date 27/1
4°C → RT + NaN$_3$ *
4°C → *
(1.1x + [illegible]) *
-20°C → 9 thaw *

② Date
Field test
created
RT 4°C *

③ (= "new" on P34, 10)
500ml batch
lot
created
4°C 24/5/95 add gold 1-2 ml
-20°C -70°C RT 4°C
* * *

thaw
make aliquots
20 µl each
at -20°C
(only 1 or 2 freeze thaw)

The unit assay *

notes from
Joel Jolas

[signature]
6-27-95

Turnover by TFI/Vent - 7 replicates of Epicenter's TFI/Vent and LTI's TFI/Vent in Epicenters SB - zero time at for stability

g N .—

pose: To establish the 3'exo activity of TFI/Vent by Turnover on gapped DNA at time zero of stability study. 7 replicates to reduce error

kground: The zero time point turnover assay has already been for Vent alone and LTI's TFI/Vent in LTI SB with 5 replicates NB 11 page 17 and 21

pro to deliver, vortex stop & vortex run - wipe tips

rials: mixA, enough for 40 rxns, each using 98ul of mix A for 23 = 1467ul H_2O ✓

~~40 x 63.786~~	=	~~2551.44ul~~ H_2O	460 5x Cheng ✓
40 x 20	=	800ul 5x Cheng	310ul act DNA
40 x 13.5	=	540ul activated DNA	11.5 dATGC-TP
40 x 0.5	=	20ul dATGC-TP, 10mM each	4.92ul α^{32}P dATP
40 x 0.214	=	8.56ul α^{32}P dATP	2253.422 6/30 AMC
		3920ul	

s	~~1-7~~	8-14	15-21	~~22-28~~	29-35
ube	~~1-21~~	~~22-42~~	43-63	~~64-84~~	85-91
		1-7	8-14		15-21
		1-21	22-42		43-~~50~~ 49
A	98ul	prewarm	to 68°C		

Vent (LTI SB) ~~2ul~~

Vent (epi SB) 2ul

/Vent-(epicenter TFI) 2ul

~~t diluted~~ (5-14-95) ~~2ul~~

2ul LTI SB = Taq SB

enz

To Page No.

ed & Understood by m ,	Date	Invented by	Dat
	6/30/95		6-29-95
		R corded by	

From Pag No.

At 5, 10, 15 min remove 10/20ul rxn to 10/20ul stop soln (p.14)
Spot 2ul on PEI plates
Spot 10ul on GFC filters (2 per rxn) + 2ul mix A 3x

tube #5

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18
19	20	21

} epicenter's TfI enzyme + Vent in epi ~~LTI~~ SB (=Tth?)

22	23	24
25	26	27
28	29	30
31	32	33
34	35	36
37	38	39
40	41	42

} LTI's TfI enz in epicenters SB + Vent = Tth

5 10 15

~~43-49~~

43-49 = no enzyme

GFC's
1-6 = 2 replicates of epicenter enz + Vent
7-12 = 2 replicates of LTI's enz in epicenter SB
13 = no enz
16-18 = 2ul mix A

T Pag

Witness d & Understo d by m ,	Date	Invent d by	Dat
	6/30/95	R c rded by Barbara Pomb	6-29-95

Project No._____

58 Book No._____ TITLE More optimization of m13 PCR s

From Pag No.— see p. 51

purpose: The PCR worked well (gave a large amount of produc
when 38 cycles were done using 400nM primer
5 u/100ul Taq
15 sec denaturat

Now we'll try to get the same good plateau yield by optimizing [primer], [enzyme], checking for anchor probe [target] & linearizing target - using just 30 cycles

program 74 1st x

expt 1. Δ [primer] from 100, 200, 300, 400 nM w/ 5u Taq, 15" dena
30 cycles

make A: 120ul 10x LTI PCR buffer
36 ul 50mM $MgCl_2$
24 ul 10mM 4 dNTP
12ul 1 pg/ul M13RF → dilute w/ TE
888ul H_2O
1080ul

rxn	1	2	3	4
A	90	90	90	90
H_2O	9	8	7	6
primer mix				

A: 50ul 10x PCR buffer ✓
15 ul 50mM $MgCl_2$ ✓
10ul 10mM 4 dNTPs ✓
5 ul 1 pg/ul M13RF ✓
395ul H_2O ✓
5 ul rTaq, 5u/ul
480ul

2ul of 370 ug/ml stock + 198ul TE → mix
2ul + 198ul TE - mix
10ul + 360ul TE ⇒ 1 pg/ul ✓

make primer mix: 6 ul 20uM anchor primer ✓
6 ul 20uM 407 primer
12ul 10uM each

To Pag

Witnessed & Und rsto d by m ,	Date 6/30/95	Invented by	Date 1-25-95
		R corded by	

g No.__	Tube	1	2	3	4
O		0	1	2	3 ✓
mer mix		4	3	2	1 ✓
]		96	96	96	96 ✓

aq] from 1 to 7 units / 100ul rxn → 400nM primers

: 80ul 10x PCR buffer ✓ dilute rTaq 5x
24ul 50mM $MgCl_2$ ✓ 6ul rTaq 54ul ✓
16ul 10mM 4 dNTPs ✓ +24ul Taq dilution buffer ✓
8ul 1 pg/ul M13RF ✓ 30ul
16ul 20uM anchor primer ✓
16ul 20uM 407 primer ✓
584ul H_2O ✓
744ul

2x ✱		5	6	7	8	9	10	11 ✓
3]		93 →						✓
H_2O		6	5	4	3	2	1	0 ✓
-Taq	14ul	1	2	3	4	5	6	7 ✓
		100ul						

target DNA, 800nM primer, 10u Taq:

2] : 35ul 10x PCR buffer ✓
10.5ul 50mM $MgCl_2$ ✓
7ul 10mM 4 dNTPs ✓
262.5ul H_2O ✓
7ul rTaq (5u/ul) ✓

✱	12	13	14	
er	4ul anchor	4ul 407	4ul anchor ✓ 4ul 407	primers are 20uM
):	94	4	0 ✓	
	92	92	92 ✓	

To Pag No.____

d & Understood by m ,	Date	Invented by	Dat
Roland	6/30/95		6-22-95
		Recorded by Carolyn Coulter	

From Page No.

Δ [Target], use 400nM primers and 5u Taq 1/100ul

for 7 rxns

[D]: 70ul 10x PCR buffer ✓
21ul 50mM $MgCl_2$
14ul 10mM 4 dNTPs
14l 20uM anchor
14l 20uM 407
462ul H_2O
7ul + Taq 5u/ul

* dilute m13 RF to 0.5 pg/ul
20ul 1 pg/ul m13 RF
20ul TC

Tube #	15	16	17	18	19	20
m13RF (0.5 pg/ul)	1	1.5	2	6	10	14
H_2O	13	12.5	12	8	4	0
[D]	86 →					

RI digestion of template:

3ul	1 pg/ul m13 RF	———	3ul
5ul	10x PCR buffer	———	5ul
3ul	50mM $MgCl_2$	———	3ul
13ul	H_2O	———	13
1ul	EcoRI 10u/ul	———	1ul H_2O
25ul			

37°C, 30'

8.3ul 8.3ul 8.3ul
91.7ul E →

37°C 30' 1pm – 1.50pm

same

21 22 23 24 25 26 To Pag

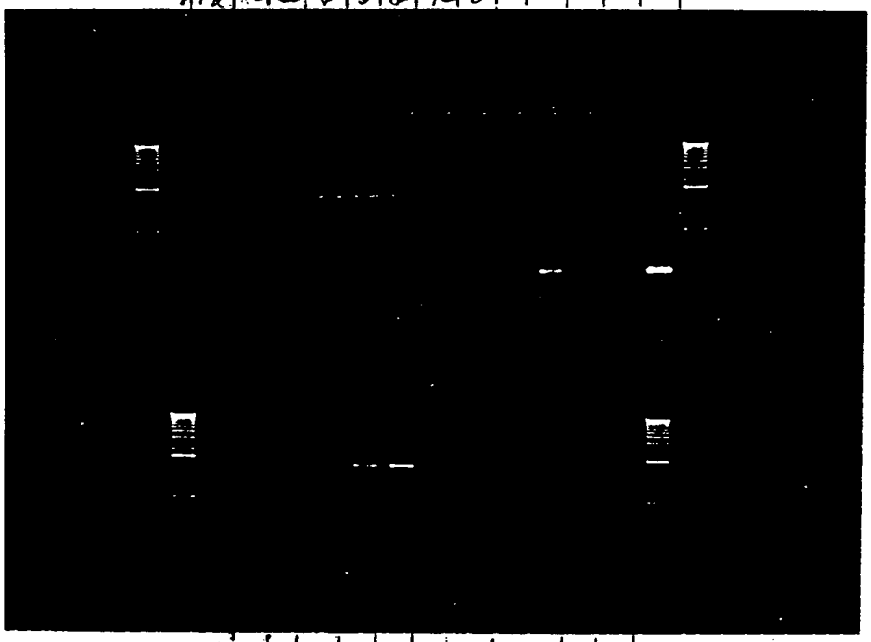
Witn ss d & Understo d by me, Dat 6/30/95

Invent d by Dat 6-29-95

R cord d by

e No.

4.6 ul	10x PCR buffer	x 7	=	32.2 ✓
2 ul	10mM 4dNTPs		=	14 ul ✓
1 ul	M13RF 1pg/ul		=	7 ✓
83.1 ul	H_2O		=	581.7 ✓
1 ul	Taq 5u/ul			7 ✓
91.7 ul				641.9



anchor & 407 are primers

To Page No.

I & Understood by me,	Date	Invented by	Date
Olaynes	6/30/95	Recorded by Carolyn Lones	6-25-15

Proj ct No._____

Book No._____ TITLE _____

From Page No.___

Results -

1. Tag is inhibitory. Best results at 1 and 2 units per 100 µl PCR.

2. Yields improve with increasing target

3. Both primers ~~must be~~ are required to make primer dimers

4. It's not hairpin substrate or one prime ~~self anneals~~ annealing to another copy of the same primer.

conclude the anchor primer should be OK for most PCRs

Page

Witnessed & Understood by m, [signature]

Dat 6/30/95

Invented by [signature]

Recorded by

Dat 6-29-95

(see P17, 9)

23 mp19 0.26 µg/µl 200 µl (158 nmol nt total)
79 nmol nt/µl
109 pmol circle/µl 21.7 pmol circle total
0.594 nmol nt/λ

23 mer 5 ng/µl 66.1 µl 43.5 pmol primer total
.66 pmol primer/µl)

266 µl

23mer/mp19 circle = 2

80°C, 5 min

cool at room temp 40 min

(for 60λ 23·mp19)
conc in 100 µl Rxn

mp19 is	10 mM Tris pH 7.4	6 mM Tris pH 7.4
	5 mM NaCl	3 mM NaCl
	0.1 mM EDTA	.06 mM EDTA

note Cheng 1X (P20, 10) =
20 mM Tricine pH 9
85 mM KOAc
2% DMSO
1.05 mM MgOAc
8% glycerol

∴ buffer in 23·mp19 will alter the reaction condition a little since 60 µl 23·mp19 is needed per 100 µl Rxn

in future need more concentrated DNA

To Page No. ______

Read & Understood by me,	Date	Invented by	Date
Bolamp	6/30/95	[signature] Recorded by [signature]	6-29-95

Results P.52

N		u	u/l relative to Taq (#10-14)	percent of zero time on P122/9
1	1597.00			
2	1752.00			
3	1760.00			
4	7709.00	6760 ave	.030	94%
5	6150.00			
6	6422.00			
7	4510.00		.025	72%
8	5085.00			
9	5662.00			
10	8620.00	8931 CPM ave	.04 (by definition)	
11	9351.00			
12	8531.00			
13	8321.00			
14	9832.00			
15	5618.00	5632 ave 5632/8931 .04	.025	.025/.023 109%
16	5895.00			
17	5384.00			
18	5128.00	5215 ave 5215/8931 (.04) =	.023 u/l	.023/.025 ≠ 100% ? P34 for start
19	5036.00			
20	5481.00			
21	3989.00	3673 ave its been at -20°C for ~5 months + 65 freeze thaws it came from tube #11 above (lines # 4-6)		54% recovery from .034 u/μl measured above (#4-6)
22	4058.00			
23	2971.00			
24	5931.00	5921 → conclude -20°C loses activity. 1 month -20°C from 5-8-95 1.1x (#15-17 above)		
25	5591.00			
26	6242.00			
27	5891.00	5712 sample from 1.1x of 5-8-95 (see tubes 15-17 above) ⇒ no activity lost for 1 month at -70°C		101%
28	5381.00			
29	5865.00			
30	5644.00			
31	5407.00	5440 started with 1.1x (stored only at 4°C) 5-8-95 (see #15-17 above)	conclude no loss of units for even 30 freeze thaws	97%
32	5271.00			
33	5362.00	5405		96%
34	5494.00			
35	5361.00			
36	5556.00	5638		100%
37	6159.00			
38	5200.00			
39	138.00	The died at 4°C off 500 col of 5-1-95 got 7.7 u/l on P.25		
40	287.00			
41	137.00			
42	1014.00	ave - BKGD = 627 CPM = 27.3 pmol	32.8 u/μl	agrees with 36 u/l
43	960.00			
44	1092.00	i.e. Flynn got and I got P.13. My 70.7 u/l of P 22 may be wrong. will conclude LW's Tne is stable until more data is available		
45	395.00	BKGD		
46	110131.00	2λ mix	68.9 CPM/pmol	
47	110429.00	2λ mix		

To Page No.______

Read & Understood by me,	Date	Invented by	Date
[signature]	6/30/95	[signature]	6-30-95
		Recorded by	6-28-95

May 8, 95

3 batch:

① 15 sample original buff. formulation — created ↓ 4°C, RT, −20°C (Date 27/1)
- 4°C → * ; RT + NaN₃ → * ; 1.1x + [illegible] *
- −20°C → 8x 9 thawed → *

② Date Field test — created ↓ RT, 4°C (circled) *

③ (= "new" on P34, 10) 500ml batch lot — created ↓ 4°C; 24/5/95 aliquoted 1-2 ml → −20°C *, −70°C *, RT, 4°C *

thaw → make aliquots 20 µl each as −20°C (only 1 or 2 freeze thaw)

The unit assay

notes from Joel Jolas

[signature]
6-27-95

ag N .— specific activity = (109,973 cpm) x $\frac{100\,ul\,rxn}{2\,ul\,spot}$ = 275 $\frac{cpm}{pmol\,(nt)}$

background = 188 cpm (5000 pmol) 4

Turnover (pmol) = $\left(\frac{cpm - background\,cpm}{specific\,activity}\right)\left(\frac{100}{2}\right)\left(\frac{20}{10}\right)$

	CPM1	
5'	774.00	$\frac{774-188}{237\ 275} \times 100 = 228\ 213$
10'	1379.00	435
15'	2170.00	721
5'	1031.00	307
10'	1588.00	509
15'	2241.00	747
5'	893.00	256
10'	1731.00	561
15'	1890.00	619
5'	788.00	218
10'	1365.00	488
15'	1836.00	599
5'	752.00	205
10'	1055.00	315
15'	1732.00	561
5'	636.00	163
10'	1140.00	346
15'	1448.00	458
5'	854.00	242
10'	1458.00	462
15	2083.00	689
	829.00	233
	1512.00	481
	2124.00	704
	980.00	288
	1612.00	518
	2249.00	749
	1182.00	361
	2028.00	669
	2271.00	757
	1040.00	310
	1816.00	592
	2521.00	848
	944.00	274
	1729.00	560
	2032.00	671
	1087.00	327
	1641.00	528
	2701.00	914
	917.00	265
	2146.00	712
	2530.00	851
	195.00	
	162.00	47
	169.00	48
	194.00	49

$\bar{X}$ 5' = 229 ± 45 (20%) → Large error, next time cut out dATP+dADP spot in order to correct for spotting error

$\bar{X}$ 10' = 436 ± 86 (20%)

15' = 627 ± 101 (16%)

$\bar{X}$ 5' = 293 ± 45 (15%)

10' = 580 ± 84 (15%)

15' = 785 ± 88 (11%)

no signal

186.00
189.00
223.00

background for turnover
n = 7 $\bar{X}$ = 188.3 ± 19.8 (~10% error)
= 6.8 pmoles

To Pag No.____

sed & Und rstood by m , Date 6/30/95 Invented by Date 4/30/95

Recorded by Carolyn Combs

Exhibit **122**
Appl. No. 09/558,421

Project ______ Book No. ______ TITLE Turnover of Tfl/Vent on 23. mp

From Page No. adjust Vf back to 266.2

23. mp19 0.~~164~~ 195 μg/μl
0.594 nmol nt/μl
P. 63

	mix A		(5.4 Rxns)
23. mp19	~~264~~ μl	264 ✓	23.7 ~~29~~ nmol nt * ~~20.2~~ μg/ml 65.6
5 x Cheng (P21, 10)	~~88~~ μl	108 ✓	* see P55 change in DNA plan
4 dNTPs 10 mM each	~~2.2~~ μl	2.7 ✓	(50 μM each)
α ^{32}P dATP 10 mCi/ml (Amersham)	~~1~~ μl	1.23	
H2O	~~76~~ μl	153.3 ✓	
	~~431.2~~	529.2	(use 98 μl/100 μl)

see p.65

Reactions

	stop tube *	①	②	③	④	⑤ (no enzyme)
		1-8	9-16	17-24	25-32	33-40
mix A		98 μl →				
						2 μl Epicentre storage buffer
Tfl/Vent = 0.18 units (epicentre Tfl 5-16-95)		2				
Vent lot #17 = (opened 2-24-95)						
0.09 u/μl = 0.18 u			2			
0.5 = 1 u				2		
2 (no dilution) = 4 u					2	
Vf = 100 μl						

note: compare to Vent alone with gapped on P 20

} dilute epicentre Tfl storage buffer

68°C in 9600, remove 8 μl to 8 μl Killing solution P.
spot 2 μl on PET and 10 μl on GFC. 13

* note ~20 μg/ml 23.mp19 is ~1/6 as much total DNA as gapped DNA in Taq unit assay (500 μg/ml) however M13 is almost all ssDNA substrate while gapped DNA may have ~20 ssDNA gaps.

at 5 10 20 40, 60, 80, 100, 120 min
* spot DuPont on PEI

To Page

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/30/95	[signature]	6-30-95
		Recorded by [signature]	

Exhibit 123
Appl. No. 09/558,421

Project No. Results of P.64

66 **Book No.**______ **TITLE** turnover on M13SS DNA

From Page No.__

	min	Turnover		(−2840 cpm, BKG) pmol	percent turnover	percent incorporation (100)(nmol incorporated / nmol input DNA)
center Tfl/vent	5	1	445.00	83	1.4	24
	10	2	1588.00	672	7.8	33
	20	3	3904.00	1866	13.6	50
	40	4	6787.00	3350	22.4	49
	60	5	6818.00	3368	21.3	52
	80	6	7009.00	3466	21.4	54
	100	7	7002.00	3462	22.4	50
	120	8	7164.00	3546	21.4	55
18u vent	5	9	333.00	25	—	0
	10	10	350.00	34	—	0
	20	11	381.00	50		0
	40	12	521.00	122	37	0.9
	60	13	832.00	282	43	1.6
	80	14	1097.00	412	45	2.2
	100	15	1474.00	615	49	2.7
	120	16	1928.00	847	53	3.2
1 unit Vent	5	17	296.00	6	2	1.0
	10	18	508.00	115	16	2.6
	20	1	1322.00	535	25	6.8
	40	2	3242.00	1524	32	13.8
	60	3	6116.00	3006	42	17.6
	80	4	8505.00	4238	45	22.1
	100	5	11510.00	5787	52	22.9
	120	6	13872.00	7004	52	27.3
4 units Vent	5	7	750.00			
	10	8	1728.00			
	20	9	4617.00			
	40	10	9106.00			
	60	11	11531.00			
	80	12	12228.00			
	100	13	12432.00			
	120	14	11890.00			
no enzyme	5	15	269.00	274 ave dAMP background		
	10	16	278.00			
	20	17	240.00			
	40	18	276.00			
	60	19	293.00			
	80	20	274.00			
	100	21	307.00			
	120	22	331.00			

note: it app[ears] there is no T/O at [...] since T/O stops [when] incorporation stops ~ 20 min

note % turnover low and mod[...] for Tfl/Vent[...] conclude most are not full [...] since low per[cent] incorp.

← about 25 [...] max incor[p...] 13.8%/5[...] (55% seen [...] at 120 min) so still [...] are not full[...]

more conclusions

1. T/O is occurring at an eff[...] high rate (~20-22%) for Tfl/[Vent] considering ~30:1 Tfl/Vent (in units)
2. it appears that substrates with 3'exo accumulate during time [...] probably at hairpins (eg M13 o[...]) rather than at full length [...] (since T/O stops shortly after incorporation stops even for V[...])

3. Incorp plateau for Vent alone (~27% incorp) is lower than for Tfl/Vent (~55%)
~~Vent gets stuck at hairpins?~~

T Pag N

Witnessed & Understood by me, [signature] | Date 7/7/95 | Invented by [signature] | Date 7-1-95
Recorded by [signature]

Results of P64

Project No. (23.7 n mol nt input ss M13 DNA)

Book No. 67

No.	Incorporation	p mol	Percent substrate copied
23	632.00		
24	37364.00		
25	37879.00		
26	106114.00		
min 27	104061.00		
28	34.00		
29	57217.00	5898	24
30	76607.00	7898	33
31	114434.00	11797	50
32	112213.00	11568	
60	120505.00	12423	
80	123172.00	12698	
100	115839.00	11942	
120	125999.00	12990	
5	1441.00	96	
10	600.00	(90)	-512 BKGD
20	1390.00	90	
40	2498.00	255	
60	4176.00	378	
80	5526.00	517	
100	6777.00	643	
120	7820.00	755	
5	2449.00	252	
10	6061.00	624	
20	15599.00	1608	
40	31777.00	3276	
60	40517.00	4117	
80	50876.00	5245	
100	52552.00	5417	
120	62653.00	6459	
53	36563.00		
54 #38	~~260.00~~		
20 55	83243.00	8582	
40 56	58704.00	6053	
60 57	82715.00	8527	
80 58	73558.00	7585	
100 1	69056.00	7119	
120 2	65008.00	6701	
	260		
60	502.00		
61	591.00		
62	450.00	ave BKGD = 512	
63	960.00		
64	307.00		
65	563.00		
66	463.00		
67	80862.00	ave 7917 ⇒	194 CPM/p mol nt
68	75044.00		
69	81620.00		

To Pag No.

ed & Understood by me,	Date	Invented by	Date
Olaup	7/7/95		7-7-95
		Recorded by Carolyn Combs	

M13 PCR system: more optimization of [primer 407], annealing temp, and [target]

g N ___

A, enough for 28 rxns: 280 ul 10x PCR buffer ✓
84 ul 50mM $MgCl_2$
56 ul 10mM dNTPs ✓
2094.4 ul H_2O ✓
5.6 ul + Taq 5u/ul ✓
2520 ul

template dilution:
to 50 pg/ul = 1 ul stock M13 RF 370 ug/mL
+ 99 ul TC
mix ✓ ✓
→ 1 ul + 73 ul TC ✓
→ 74 ul of 50 pg/ul
→ to 10 pg/ul
8 ul + 32 TC ✓
40 ul 10 pg

15 53° 10:50 am
16 55° 11:02 - 1:15
SGI 57°

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

1 - 3 4 - 6 7 - 9

3 RF 3 ul of 10 pg/ul 9 ul 10 pg/ul 6 ul of 50 pg/ul ×

mer mix + 12 ul 18 24 12, 18 ul 24 12, 18, 24 ul ✓
um anchor
um 407
anchor 407 ✓

O 15 ul 9 ul 3 ul 9, 3 ul 0 12 ul, 6 ul, 0 ul ✓

A 270 ul → 270 ul → 270 ul →
300 ul →

divide into 3 tubes, 1 for each annealing temp. a, b, c

= 53° 10 pg M13RF 4 a 53° 30 pg M13 7 a 53° 100 pg M13
55° w/ 400nM primers b 55° 400nM primers b 55° 400nM p
57° c 57° c 57°

= 53° 10 pg M13RF 5 a 53° 30 pg M13 8 a 53° 100 pg M13
55° w/ 600nM primer b 55° 600nM p b 55° 600nM p
57° c 57° c 57°

6 a 53° 30 pg M13 9 a 53° 100 pg M13
53° 10 pg M13RF b 55° 800nM p b 55° 800nM primers
55° w/ 800nM primers c 57° c 57°
57°

T Pag No. ___

d & Und rstood by me, Date 7/7/95 Inv nted by Date 7/5/95
R corded by Carolyn Combs

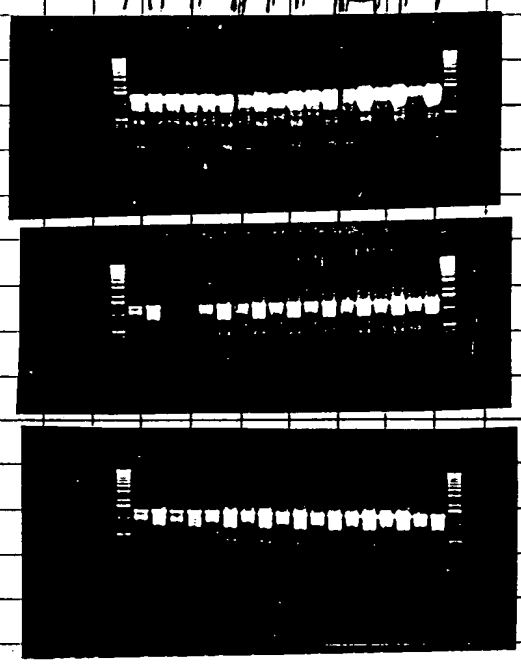
Project No._____

70 Book No._____ TITLE _____

From Page No.__

1% agarose gels: 6 g agarose
600 ml 1x TAE
~~40~~ 36 ul 10 mg/ml EtBr

M13RF:	10 pg	30 pg	100 pg
primer (nM)	400	600	800
cycles	30	35	



top
57°C C 1-9
55°C B 1-9
53°C A 1-9
bottom

Conclusions

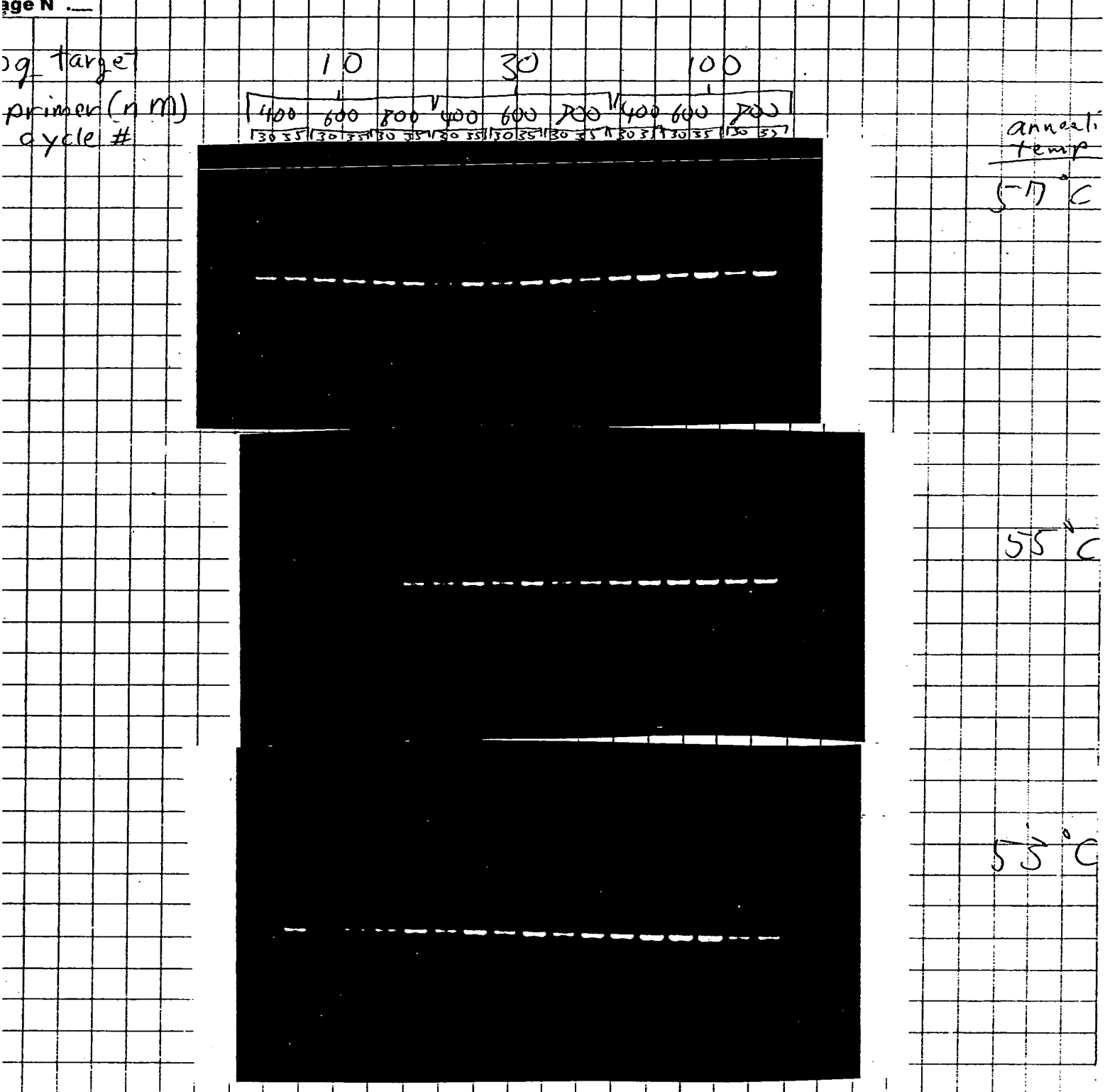
1. no advantage to >400 nM primer
2. more target improves yield and specificity
3. 57°C is most specific

Carolyn Compton 7/6/95

To Page

Witnessed & Understood by me,	Date	Invented by	Dat
D Bolano	7/7/95		7/5/95
		Recorded by Paul Compton	

Page N ___



To Page No._____

ed & Understo d by me,	Date	Invented by	Date
	7/7/95	R corded by	7-6-95

From Page No.

title: m13 PCR system: 4 annealing temperatures and 4 primer sets

53°C, 55°C, 57°C, 59°C anchor + 6681, 7069, 407, 806

purpose: We have established optimal [template] & [primers] for anchor + 407. Now we'll optimize annealing temp and cycle # for the other primers 6681, 7069, 806. ~~+ redo 4~~ assuming that these other primers will work well with the [template] & [primer] that worked well w/ 407. Later, we'll titrate [Taq] & [Tne] for these primers.

background:
- 59°C has not been tried before w/ any primers
- in an earlier expt 57°C worked best for 407
- [template] = 100 pg / 100ul rxn - found to be best for 40
- [primer] = 400 nM - found to be best for 407
- [Taq] = 1u/100ul rxn, more was inhibitory for 407
  2u/100ul rxn will be tried for 806 which me
  the longest product
- expected product sizes:

anchor + 6681 → 380 bp
+ 7069 → 768 bp
+ ~~+356~~ 407 → 1356 bp
+ 806 → 1755 bp

Carolyn 7/6
Combs

materials: m13RF 50 pg/ul, diluted on: ~~7/6~~ 7/6/95 NB11 p.

To Pag

Witness d & Understo d by m, Dat 7/7/95
Invented by
Date 7/95
R c rded by
7/6/95

ocedure:

ake a master mix for 21, 100ul rxns - containing everything but the primers:

(A)
- 210ul 10x PCR buffer
- 1654.8ul H_2O
- 63ul 50mM $MgCl_2$ C_f = 1.5mM
- 42ul 10mM dNTP's C_f = 200uM
- 42ul M13mp19RF 50ng/ul stock
- 42ul anchor primer, 20uM stock C_f = 400nM (C_f = 100ng/100ul rxn)
- 4.2ul Taq 5u/ul C_f = 1u/100ul rxn

2058ul

remove 441ul and add 0.9ul Taq (5u/ul) - for 806 primer
w/ 2u Taq/100ul rxn

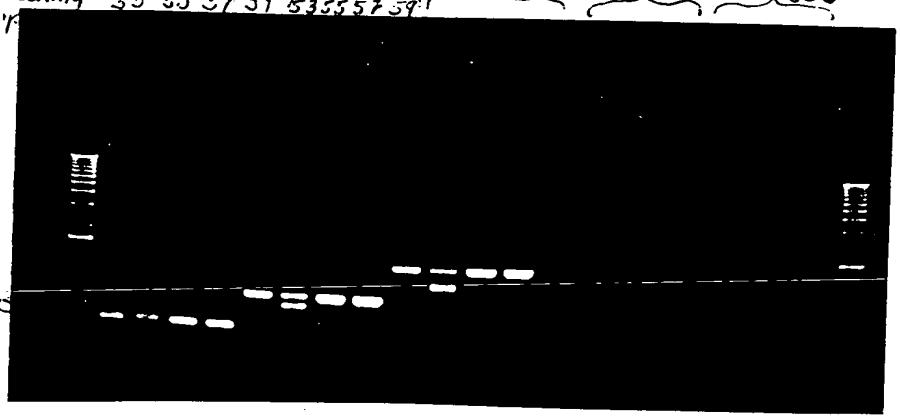
s (1.5mL)	1	2	3	4	5
ner, 20uM stock	8ul 6681	8ul 7069	8ul 407	8ul 806	8ul 806 (B) →
	392ul				
	400ul				

ivide into 4, 100ul aliquots in 9600 PCR tubes
nd put each tube in different 9600's set to different
nnealing temperatures 53° - Lab 15
55°C - Lab 16
57°C - SGT
Tanlyn Combs 7/6/95 59°C - Lab 14 * note that 30 cycle aliquots where taken during ramp to 94°C

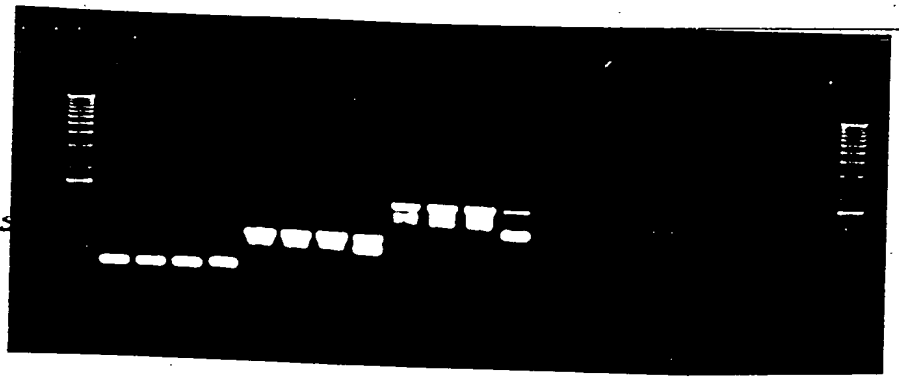
Pause the 9600's during later part of the 70°C, 2' extension to withdraw 25ul samples at 25, 30, 35 cycles - + 2.8ul Blue Juice

d & Understood by m,	Date	Invented by	Date
Polan	7/7/95	Recorded by Carolyn Combs	7/6/95

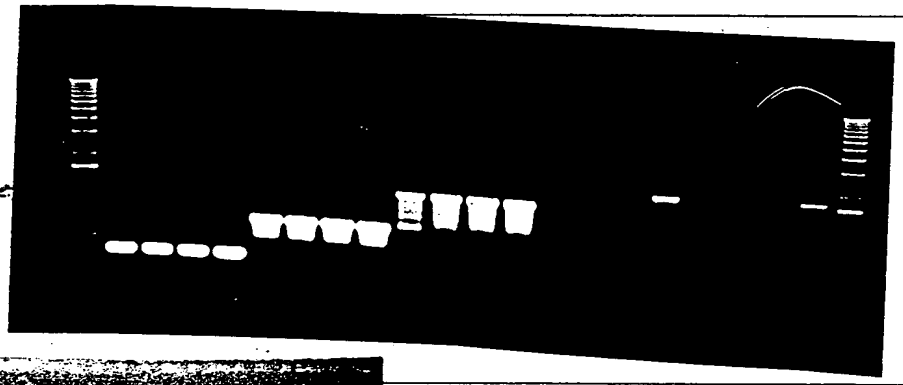
primer 6681 7069 407 1u Taq 806 2u Taq 806
annealing temp 53° 55° 57° 59° 53 55 57 59



25 cycles



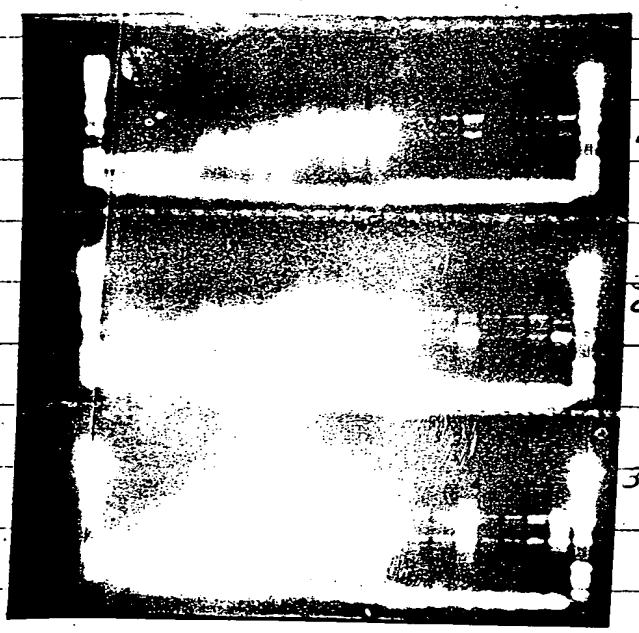
30 cycles



35 cycles

Note: 59°
cycles - I
9600 during
to 94°C so
product is,
ssDNA-acc
for the 2.
in 7069 & 4

Carolyn Comb
7/6/95



25 cycles

30 cycles

35 cycles

- 59°C annealing temp for 25 cycles gave a good produc yield and the least nonspecifi products for primers 6681, 7069, 407
- Try higher annealing temps fa primer 806 because product i beginning to come up at 59°C

Witnessed & Und rsto d by me,	Date	Invent d by	Date
BPolump	7/7/95		7/6/95
		R corded by Carolyn Comb	

m13 PCR system: titration of [Tne] and [Taq] with 6681, 7069, & 407 primers - 3 cycle #s

g N__

rpose: 1) To optimize the [Taq] -> 1u/100ul rxn was previously found to be inhibitory, so lower conc. will be tried - 0.25 / 1u.

2) To see if Tne can synthesize any of the products expected w/ 6681, 7069, &/or 407 primers + anchor primer - no product was made (p.44) when 5u Tne/100ul was tried. The [template] and [primers] that were optimal for Taq will be used in the Tne PCR rxns. .1 - 1 u will be tried

- 25, 30, 35 cycle samples will be taken
- 59°C annealing temperature, 400nM primers, 100pg/100ul rxn m13 target

terials:

mix [A] w/ 6681, for 11.5 rxns

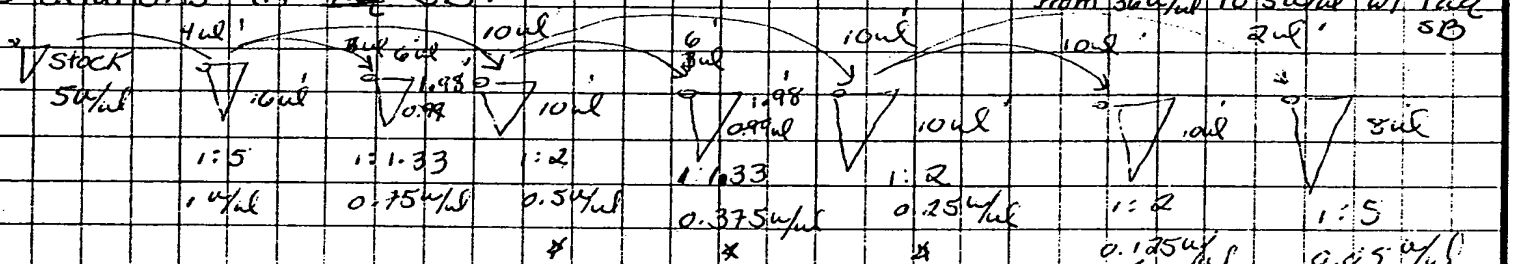
885.5 ul	H_2O	✓
115 ul	10x PCR buffer	✓
34.5 ul	50mM $MgCl_2$	Cf = 1.5mM ✓
23 ul	10mM dNTPs	Cf = 200uM ✓
23 ul	20uM anchor ✓	→ Cf = 400nM each
23 ul	20uM 6681 primer ✓	
23 ul	M13RF 50 pg/ul ✓	Cf = 100pg/rxn
1127 ul		

↓ see p. 69 for dilution of stock to 50 pg/ul for FAS 701

mix [B] same as [A] but use 23ul, 20uM 7069 primer ✓

mix [C] same as [A] but use 23ul, 20uM 407 primer ✓

de dilutions in Taq SB: RT, rinse pipet, vortex 2sec — 5/7/95 Liz Flynn diluted w/ Taq SB



also do same dilutions w/ Taq in Taq SB - only ✱ dil will be used

top soln = 100mM EDTA → 80ul 0.5M EDTA + needed to kill exo
8x Blue Juice + 320ul 10x Blue Juice ice won't kill it
400ul

To Page No.____

d & Understood by me, Polano	Date 7/7/95	Invent d by	Date 7/6/95
		Recorded by Carlos Flores	

Project No._____

76 Book No._____ TITLE _____

From Page No.__

rxn #	1	2	3	4	5	6	7	8 → 14	15 - 21
	98ul [A]							98ul [B]	98ul [C]
1% Tne	3ul							same as 1-7	same
0.75% Tne		2ul							
0.5% Tne			2ul						
0.375% Tne				2ul					
0.25% Tne					2ul				
0.125% Tne						2ul			
0.05% Tne							2ul		
	100ul								

*remove 25ul rxn after 25, 30, 35 cycles to 2.8ul Blue Juice

note: 59°C annealing temp, 10:40 am - 2

94°C
94°C, 15"
59°C, 30" > Lab 15 program 74 9600
70°C 2' extension
4°C final

rxn #	22	23	24	25	26 - 29	30 - 33
	98ul [A]				98ul [B]	98ul [C]
0.5% Taq	2ul				2	2
0.375% Taq		2ul			2	2
0.25% Taq			2ul		2	2
0.125% Taq				2ul	2	2
0.05% Taq						

30 cycles only

~~** remove 25ul to 2.8ul Blue Juice~~

* remove 25ul of Tne rxns after 25, 30, 35 cycles during last part of 2min, 70°C elongation into 4ul stop soln in microtiter plate. The final [EDTA] = 10mM
[Blue Juice] = 1.1X

** remove 25ul of Taq rxns after 30 cycles + 4ul stop soln

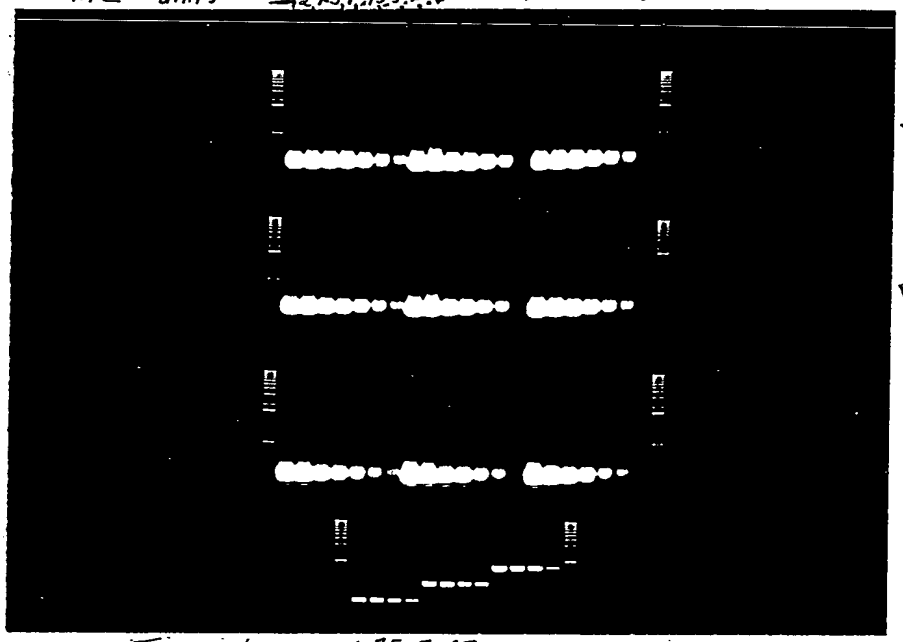
To Pag N

Witnessed & Understood by m ,	Date 7/7/95	Invent d by	Date 7/7/95
Molano		R c rd d by Sandra Pomb	

'ag N .__ Results

pected product size	380 bp	768bp	1,356 bp
primer #	6681	7069	407

Tne units 1.25, 1, .75, .5, .25 ← same → same



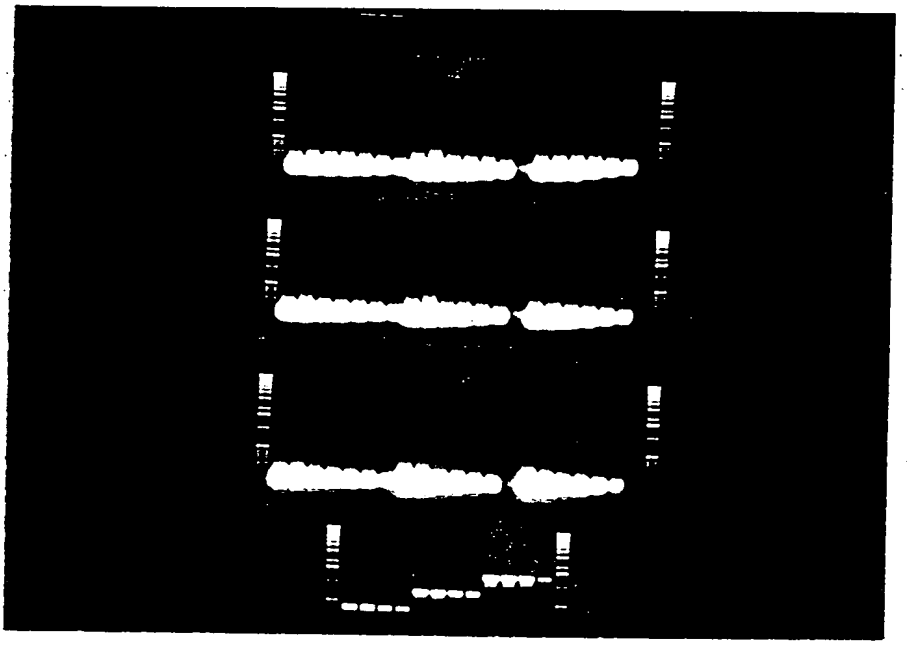
Taq units 1, .75, .5, .25

1) Taq made the expected specific products. 1 u/100 ul rxn Taq was optimal

2) Tne didn't make any specific products - only primer-dimers more Tne gave more primer dimers - try primer extens exp - low pol rate?

← 35 cycles
← 30 cycles
← 25 cycles
Tne

← Taq 30 cycles



longer exposure of same gel

To Page N .____

ed & Understo d by me,	Dat	Invented by	Date
	7/13/95	Recorded by Carolyn Lamb	7/7/95

[redacted]

PCR with old & new Taq

Exhibit **127**
Appl. No. 09/558,421

78 **Project No.**______ **Book No.**______ **TITLE** GAPDH pUC

From Pag No.__

	A	B	10 R
5x Cheng (no dNTPs) (P2, 10)	100	100	
10mM dNTPs	10	10	(200 µM
Human spleen genomic DNA (HS #2 19/4) 80 ng/µl	12.5		(100 ng/5
GAPDH(+) 2112, 10µM	20		Cf (400 nM
GAPDH(-) 2113 10µM	20		Cf Mg for
Mg OAc 100mM	—	2.5	Cf=1. for B
pUC DNA XmnI 25 pg/µl		10	
2836, 10µM		20	
2837, 10µM		20	
H_2O	317.5	317.5	
	480	480	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A	48 →																	
B										48 →								
Tne 5-7-95 (Liz)																		
0.5	2									2								
1.5		2									2							
2			2									2						
				2 2.5									2 2.5					
Tne 53u/µl Adam 3-2-95 diluted 2u/µl					1	2								1	2			
Taq 2u/µl							1	2								1	2	

all dilu(tions) made with ~~Taq dilution~~ Taq storage buffer

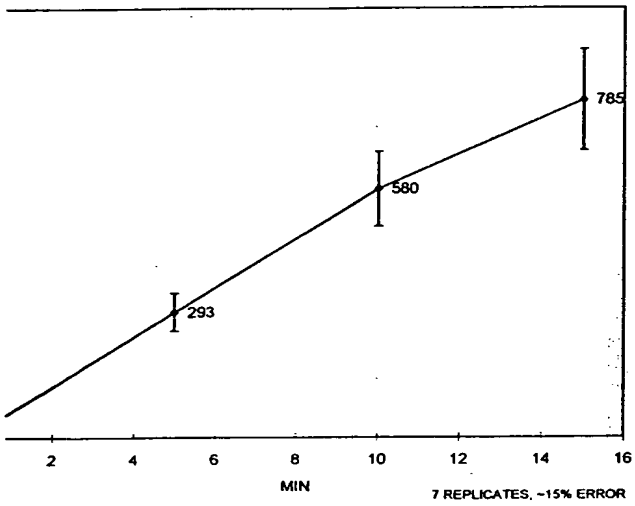
T Page N

Witness d & Understo d by m ,	**Dat**	**Inv nted by**	**Dat**
[signature]	7/14/95	[signature]	7-10-95
		R cord d by [signature]	

g N .__

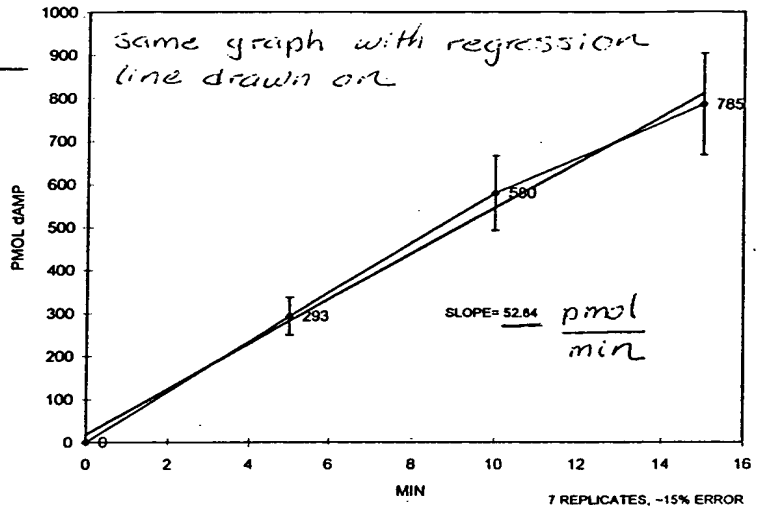
Sheet3 Chart 1

TS Tfl/Vent IN EPICENTER'S SB: TURNOVER AT TIME ZERO IN STABILITY STUDY (6/28/95)



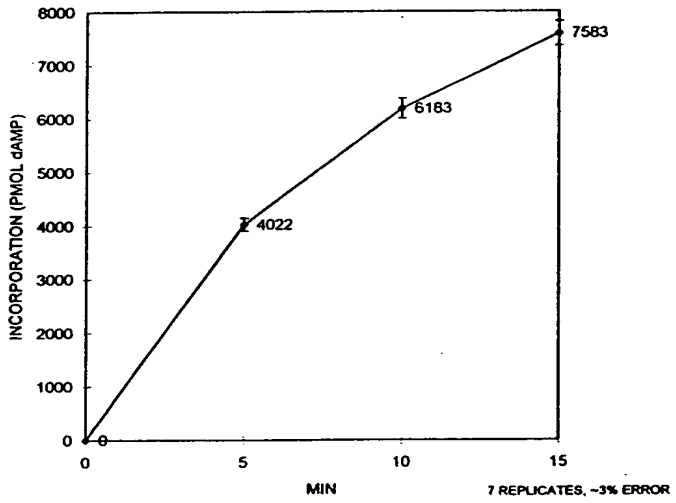
Sheet3 Chart 1

LTI'S Tfl/Vent IN EPICENTER'S SB: TURNOVER AT TIME ZERO IN STABILITY STUDY (6/28/95)



Sheet4 Chart 1

LTI'S Tfl/Vent: POLYMERIZATION AT ZERO TIME POINT IN STABILITY STUDY (6/28/95)



calculations:

To Pag No.____

ed & Und rstood by me,	Dat	Invented by	Date 7/11/95
		Rec rded by Carolyn Combs	

Project No. _____ Book No. _____

M13 PCR: Titration of Tne & Taq in (Cheng) buffer

Page No. _____

purpose: Can we make the 380bp m13 PCR product w/ Tne? Earlier p. 77 w Tne failed to make this product in 10x PCR buffer.

4 for ~~17~~ rxns, 100ul per rxn: 340 300ul 5x Cheng buffer
34 30ul 10mM dNTPs C_f = 200uM
34 30ul 50 pg/ul m13mp19 RF* in TC C_f = 100pg
34 30ul 20uM anchor primer
34 30ul 20uM 6681 primer C_f = 400nM
1190 ul H_2O

1666ul +476ul

ions of Taq & Tne (5/7/95 Liz) in Taq storage buffer (SB)

15ul + 10.05ul TaqSB → 3u/ul 1.67x

20ul + 4ul TaqSB → 2.5u/ul 1.2x

20ul + 5ul TaqSB → 2u/ul 1.25x

20ul + 6.6ul TaqSB → 1.5u/ul 1.33x

20ul + 10ul TaqSB → 1u/ul 1.5x

10ul + 10ul TaqSB → 0.5u/ul 2x

10ul + 10ul SB → 0.25u/ul 2x

10ul + 10ul SB → 0.125u/ul 2x

1 2 3 4 5 6 7 8 | 9 10 11 12 13 14 15 16

98ul A →

2ul +Taq (2 2 2 2 2 2 2 2)

2ul Tne (5-7-95 Liz) (2 2 2 2 2 2 2 2)

100ul

1st 1 min 94°C
cycle: 94°C 30" denat.
55°C annealing temp 30"
72°C exten 2'
remove ~~25~~ 35ul aliquots after 25, 30, 35 cycles
program 76
1pm – 4pm
16 x 3 = 48

* remove 25ul p. 79
+ 3ul STOP soln (w EDTA)
* run 25ul

To Page No. _____

Read & Understood by me, [signature]

Date 4/14/95

Invented by [signature]

Recorded by [signature]

Date 7/11/95

Project No.

84 Book No. TITLE M13 PCR: 3 small products w/ Tne

From Page No.

purpose: To determine if Tne can make the 3 smallest m13 PCR produ
380 bp, 768, 1356 bp, ~~380~~ using the conditions whic
worked for gapDH & pUC p.80 { Cheng buffer, 400uM p
35 cycles { 55°C annealing temp
94°C 30" denat, 100 pg/r
72°C 2' extention

If the products are made, we can use these conditions for
~~materi~~ m13 primer extention experiments

materials: mix for 15, 50ul rxns

[A] 150ul 5x Cheng
510ul H_2O
15ul 10mM dNTPs
15ul 50pg/ul m13mp19 RF
15ul 20uM anchor primer
705ul

[B] = 235ul A + 15ul 20uM 6681

[C] = 235ul A + 15ul 20uM 7069

[D] = 235ul A + 15ul 20uM 407

dilutions of Tne (5/17/95 Liz) in Taq storage buffer:

5u/ul stock → 10ul → +28.3ul TaqSB 33.3ul → 1:3.33x, 1.5u/ul → 20ul → +10ul TaqSB 30ul → 1:1.5, 1u/ul → 20ul → +20ul TaqSB 40ul → 1:2, 0.5u/ul → 20ul → +20ul TaqSB → 1:2, 0.25u/ul → 20ul → +20ul Taq → 1:2, 0.12

rxn	1	2	3	4	5	6	7	8	9	10	11	12	13
[B]	48	48	48ul										
[C]				48ul →									
[D]									48ul →				
1.5u/ul Tne				2					2				
1u/ul	2				2					2			
0.5u/ul		2				2					2		
0.25u/ul			2				2					2	
0.125u/ul								2					2

→ start rxns on ice w/ en

Result on p.86
Only the 380k product was made.

T Page N

Witn ss d & Understood by me, DD Dolan

Date 7/14/95

Invent d by

Rec rded by Dawson Pimb

Dat 7/12/95

^{32}P 23 · M13 for Tfl / vent

Project No.______ Book No.______

Exhibit 131
Appl. No. 09/558,421

same experiment as P. 64 except ^{32}P 23 instead of ^{32}P dA...
increasing T/O with time (P 64-68) due to strong pause sites
visible on an agarose gel ?

5 ng/λ 23 mer	35 μl	175 ng
5x kinase buffer	10.2 μl	25 pmol 23 mer
PNK	1.2 μl	
γ ATP 10 mCi/ml 7-14-95	5	
	51.4 μl	

37°C, 30 min → 55°C, 5 min

	51.4 μl	
H_2O	42.6	
M13 mp19 0.2 μg/λ .79 nmol nt/λ .109 pmol circle/λ	106	11.5 pmol circle total
	200	

(23 mer / circle = 2)

70°C, 5' cool slow

[f] = 0.41 nmol nt M13/μl

it will be ~23.7 total nmol nt / 100 μl Rxn
same as for P. 64

To Page No.______

Read & Understood by me, Polanys Date 7/14/95

Invented by [signature] Date 7-13-95

Recorded by RJ

Project No.______

Book No.______ TITLE ______________________

From Pag No.__

	A	3.5 Rxns
32P23mer-mp19 (P87)	171.1	
0.41 nmol nt/μl		
.0566 pmol circle/λ		
5X Cheng (no dNTPs)	70	
P21,10		
10mM dNTPs	1.75	Cf = 50μM
H2O	100.15	
	Vf = 343	(use 98μl/100μl R

	①	②	③	
A	98μl	→	→	
Tfl/Vent (epicenter Tfl) 5-16-95	2			← (1.88u total in its only 0.94 (epicenter units) due with Vent by Ni
Tfl lot 31010A-502 1u/λ (epicenter units)		1.88μl		(1.88 unit Tfl
Vent 2u/μl lot #17 opened 2-24-95			2	(4 units) ... more Vent than in Tfl, in order to get full leng products)

68°C. Remove 8μl at 1, 2, 5, 10, 15, 20, 40, 60, 9 to 1μl 10X 'blue juice' mM E

run on agarose (same as P56, 9)

To Pag N

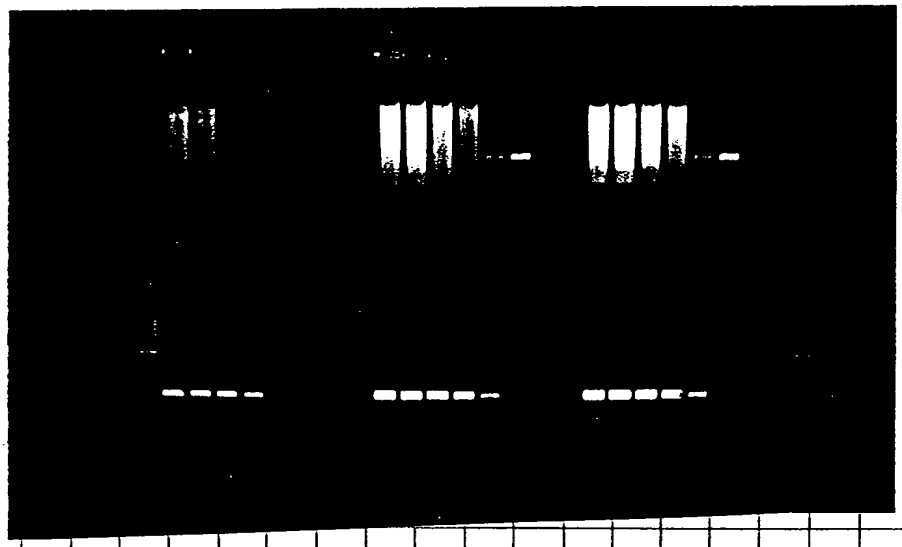
Witnessed & Understood by me, D Polaup | Dat 7/14/95 | Inv nted by [signature] | R c rd d by | Dat 7-14-95

om Page No.

1% agarose gel

top empty

9-16	9-16	9-16	Tne 9-16 decreasing enz
25	30	35	
1-8	1-8	1-8	Taq 1-8 decreasing enz - run into gel 1st
35	30	35	Tne from p. 80



same photo as p. taken from a furth distance to capt the ~~with~~ bottom of gel

To Pag N

tnessed & Understood by m , Date 7/14/95

Inv nted by

R c rded by Pauline Pinto

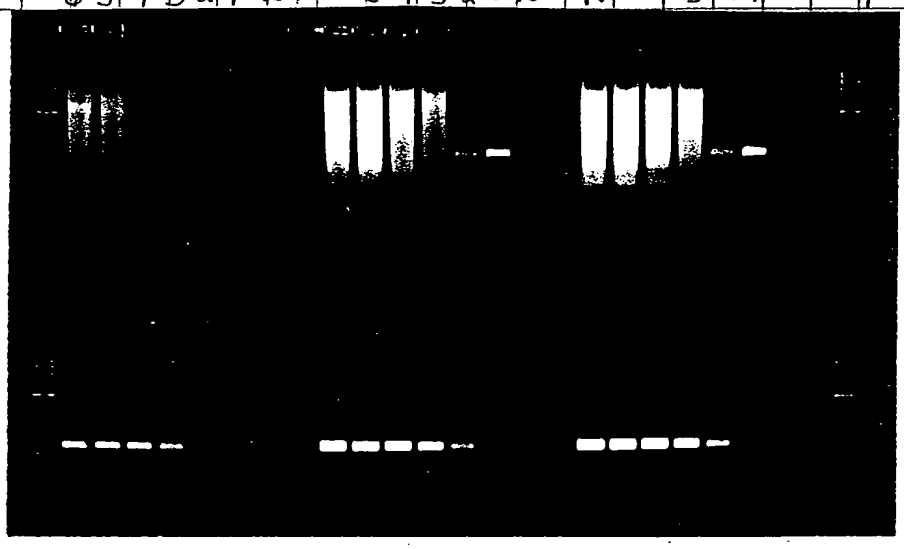
Date 7/14/95

Page No.__

?heng buffer, 380bp product expected

25 cycles | 30 cycles | 35 cycles

units 6 5 4 3 2 1 .5 .25 | 6 5 4 3 2 1 .5 .25 | 6 5 4 3 2 1 .5 .25 | 1 pool rxns



Tne

rTaq

- 1 unit Tne is optimal, 35 cycles ~~2~~ 1.5
- 6 units rTaq gave the most product for all cycle #'s

To Page No.____

ed & Understood by me,	Date	Invented by	Dat
Polcyn	7/14/95	Recorded by Carolyn Combs	7/14/95

213 PCR: Δ PCR buffer into Cheng buffer

Exp No.

purpose: To find out what component(s) of Cheng are important for making the broad smear (vs narrow, low mw smear made w/ PCR buffer)

1) 3g agarose
6mL 50x TAE
294mL H_2O
300mL wt 514.59 w/ magnet
+ 20ul EtBr

a/s: [A] for 35 rxns = 35 ul 20uM anchor primer
35 ul 20uM 6681 primer
35 ul 10mM dNTPs
910 ul H_2O
1015

→ [B] w/ target
507.5 ul of [A]
+ 17.5 ul 50pg/ul m13 RF

→ [C] no target DNA
507.5 ul of [A]
+ 17.5 ul H_2O

*Nens 2/10/95

[D] = mix of Tricine 200mM + MgOAc 10.05 mM to add KOAc to = 10x ~~working~~ stock
200 ul of 1M Tricine pH 9 (from Nen)
+ 10.05 ul of 1M MgOAC
+ 789.95 ul H_2O
1mL

[E] = mix of 166.6mM Tricine pH 9, 708 mM KOAc, 8.75mM MgOAc
is 8.33X stock
166.6 ul 1M Trine pH 9
354 ul 2M KOAC
8.75 ul 1M MgOAC
470.65 ul H_2O

Tne (5-7-95) Liz 5+ul diluted to 0.2+/ul w/ Taq storage buffer 148.8 ul H2O + 6.2 ul Tne
τ 12/7/95

Mix:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 → 28
	30ul [B] target														30ul [C] no target
Buffer	5														
$MgCl_2$	1.5														same as 15-28 without target
		5	5	5											
dNTPs		10													
KOAc			2.35	5.88											
					6	6	6	6	6	6	6	6	6	6	
Glycerol					2	5	8					8	8	8	
H2O	8.5	0							.5	.75	1	.5	.75	1	
	~~8.5~~	~~7.65~~	~~4.12~~	9	7	4	8.5	8.25	8	0.5	.25	0			
Tne	5														
	50ul														

To Page No.

Read & Understood by me,

Date 7/14/95

Invented by

Recorded by

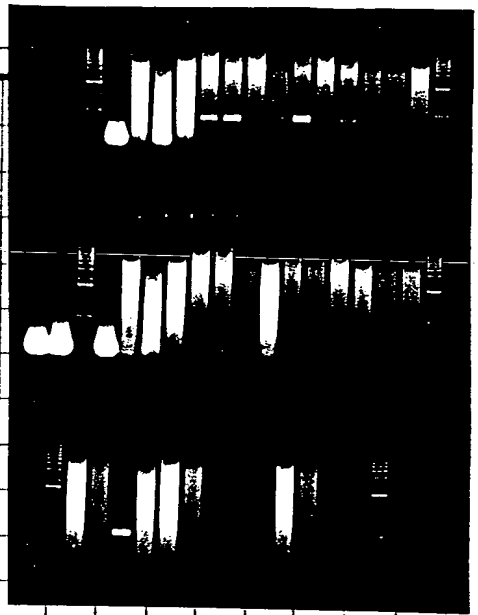
Date 7/14/95

Project No._____

Book No._____ TITLE _____

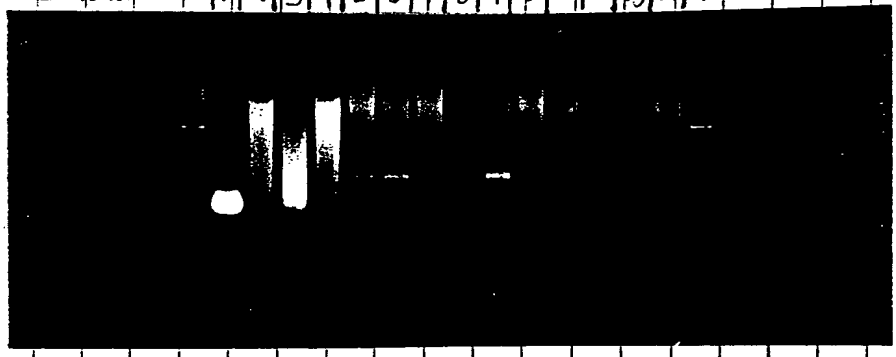
From Page No.__

Buffer components	1x buffer	mM													chem
Tris pH 8.4	20														
KCl	50	50													
$MgCl_2$	1.5														
Tricine pH 9		20													
MgOAc		1.05													
KOAc			20	50	85										
glycerol						2%	5	8			3	8	8	8	% ← really is % glycerol due to contribution from enzyme
DMSO									1	1.5	2	1	1.5	2	%
Lane #		1	2	3	4	5	6	7	8	9	10	11	12	13	14



← 1Kb ladder

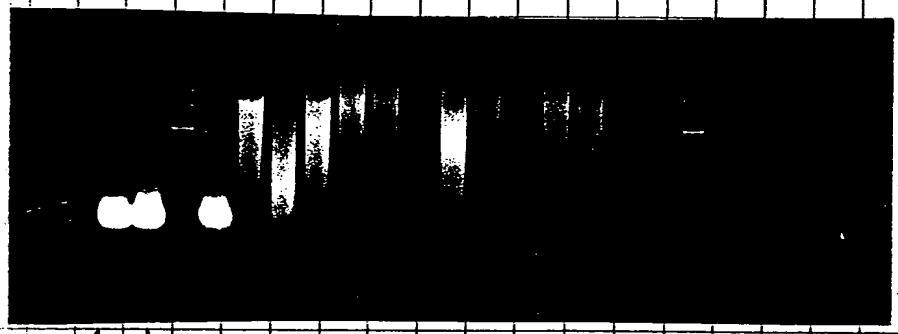
← expt on p.8



c.conds 7/14/95

+m13 template + 2 primers for 380bp produc

* Note, the enzym brings glycerol i rxn, 50 [glycero 7, 10, 13%



c.conds 7/14/95

—m13 template + 2 primers

no template, only

1 anchor primer alone

1 (6681 primer alone

1) The transition from "primer dimer" (smallest band) to smear occurred when Tricine pH 9 + MgOAc 1.05mM was substituted for Tris pH 8.4 + MgCl
2) Product was made when 85mM KOAc was added to the tricine MgOAc buffer - glycerol + DMSO were not required to make produc
3) Less glycerol + DMSO resulted in more product synthesis
4) The smears are present in the plus and minus target lanes
5) The "primer dimer" is made when either primer is present alone if both primers are present as long as PCR buffer is used

To Pag

Witn ssed & Understood by m ,	Dat	Invent d by	Dat
Polanyi	7/14/95		7/14/95
		Record d by	

Project No.______
Book No.______ TITLE ~~Tne on P. 86~~ ?

is 32P primer incorporated into either the "primer dimer" or smear see

From Page N ___

	(−Target) A	(+Target) B	(85 mM KOAc) C	10 Rxns
1 M Tris pH 8.4	10			20 mM
3 M KCl	8.33	8.33	—	(50 mM)
50 mM MgCl2	15			(1.5 mM)
1 M Tricine pH 9		10	10	20 mM
MgOAc 50 mM		10.5	10.5	(1.05 mM)
M13 RF 50 pg/µl	—	10	10	(50 pg / 50 λ PCR)
10 mM dNTPs	10	10	10	
			21.25 ←	2M KOAc
H2O	356.7	361.2	~~350~~ 347.25	
VF =	410	410	410	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	41 µl →																
B								41 µl →									
C																	41
6681 10 µM	2		2		2		2		2		2		2		2		2
6681 10 µM				2				2				2				2	
301 10 µM		2		2		2		2		2		2		2		2	
16301 10 µM			2				2				2				2		2

Cf = 400 nM

Tne 5-7-95 (2) 5 µl → (0.2 U/µl diluted in Taq SB)

note for 3 µl 8% PAGE its 0.08 µl 32P primer

35 cycles as per P 86

mix 20 µl PCR with 2 µl BJ, 100 mM EDTA load 10 µl on 0.8% Ag as per P 56, 9 — 150 V start ~2 PM (?)
Process as per AK 6 ... 7 last 4:30

To remaining 30 µl PCR add 15 µl cycle seq stop, load 5 µl on 8% PAGE (wells 1–17 are PCR reactions

for sequence Rxn use 32P 6681 · mp19 ssDNA (PT1) as per (P 27, 4)

To Pag N

Witnessed & Understood by me, [signature] Date 7/14/95
Invented by [signature] Date 7-14-95
Recorded by

^{32}P 6681 and ^{32}P 6301 anchor

No. (Can use P150, 9)

	①	②		
100 µM 6301 (anchor)	1.5 µl		✓	Cf = 10 µM Cf
100 µM 6681		1.5 µl	✓	
^{32}P γATP 10 mCi/ml	10	10	✓	
f = 7-14-95				
PNK, H$_2$O	1	1	✓	Cf of 1X Kinase buffer
5X Kinase buffer	3.1	3.1	✓	35 mM Tris pH 7.6
	15.6 µl	15.6		50 mM KCl
				5 mM MgCl$_2$
µM 6301 cold	78.5			
µM 6681 cold		78.5		
	93.98			

e dilute that primer with 5 parts cold primer
∴ contribution to PCR of p90 is:

	primer	in PCR
MgCl$_2$	0.83	0.053
Tris pH 7.6	1.8 mM	0.23 mM
KCl	8.3	0.53

^{32}P 6681 · mplg for sequence

mplg 0.26 µg/µl 0.109 pmol circle/1 10 µl (1.09 pm circle

^{32}P 6681 10 µM primer 1 10 pm prim

70°C cool slow

for 3µl on PAGE is ~0.04 µl primer

To Page No. _____

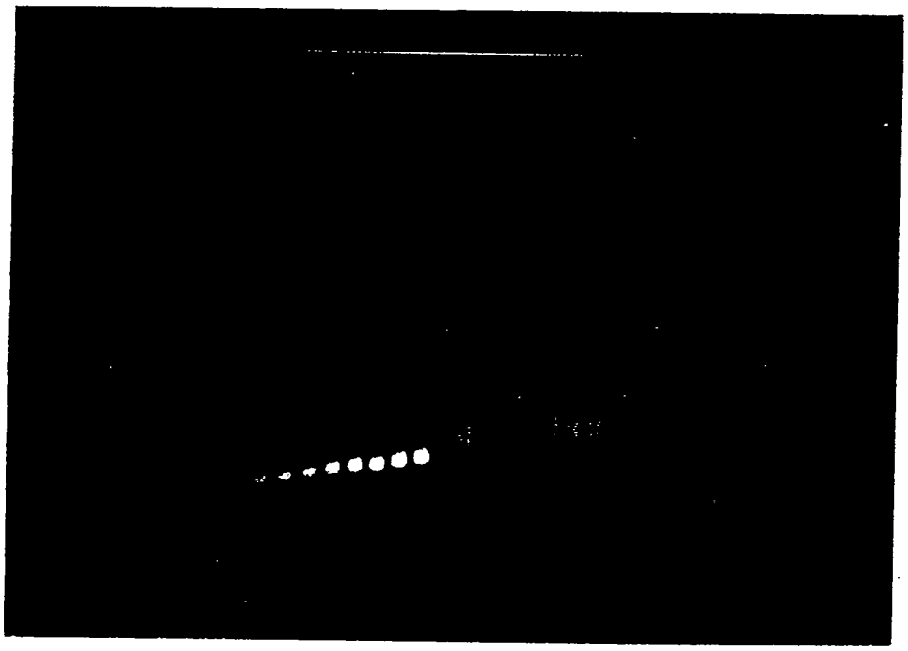
d & Understood by me,	Date	Invented by	Date
Polanp	7/14/95	Recorded by	7-14-95

From Page

1.5 mM $MgCl_2$ 1.05 $MgOAc$

Tris pH 8.4 Tricine pH 9

KOAc 85 mM



PCR

Tne

32P primers

→ 0.8 % Agarose → EtBr

→ Autorad

→ 8% PAGE (denaturing) → Autorad

Witn ssed & Underst od by m , | Dat | Inv nt d by | Dat 7-14-95

R c rded by

PAGE 93 OF NOTEBOOK WAS BLANK

Project No._____

94 Book No._____ TITLE Primer dimer/smear transition: effect of Tris 8.4/Tricine 9, [Mg], MgOAc vs MgCl

From Page No.___ 7/14/95 purpose: To determine which buffer component(s) were resp

1) For the transition seen from "primer dimer" to "smear" on p.86 1q
The "primer-dimer" condition was 20mM Tris 8.4
50mM KCl
1.5mM $MgCl_2$

what comp is causin transitio

The "smear" condition was 20mM Tricine pH 9
50mM KCl
1.05mM MgOAc

? Tris 8.4 vs [Mg] MgOAc

2) Is the "smear" or "primer dimer" due to DNA contamination of the prep? do rxns w/o D

materials:

180.ul 0.2 u/ul Tne (5-7-95 Liq) → 7.2ul Tne stock 5u/ul
+ 172.8ul Taq storage buffer

! 100mM Tris 8.5 * note this is a change from the original 8.4 "
dimer" condition 100ul 1M Tris 8.5 (RL)
900ul H_2O

! 100mM Tricine pH 9 100ul 1M Tricine pH 9 (Nen 2/10/95)
900ul H_2O

~~5.25~~
~~10.5~~ mM $MgCl_2$ ~~210~~ul 50mM $MgCl_2$ — 105ul
~~790~~ul H_2O — 895ul

! ~~7.5~~
~~15~~ mM $MgCl_2$ ~~300~~ul 50mM $MgCl_2$ — 150ul
~~700~~ul H_2O — 850ul

5.25
~~10.5~~ mM MgOAc 10.5ul 1M MgOAc ~ 5.25 — 10.5ul 5.25
989.5 ul H_2O 1989.5ul 994.7
2mL

7.5
~~15~~ mM MgOAc 15ul 1M MgOAc — 7.5ul 7.5ul
485ul H_2O — 1985ul 992.5ul
2mL

T Pag N

Witn ss d & Und rst od by me, Dat 7/24/95 Inv nt d by Dat 7/14/95
Rec rd d by Carolyn Pomb

e N .__

25:

A = 360ul 10mM dNTPs ✓
29.88ul 3M KCl ✓
726.12ul H_2O (1st) ✓
792ul

[C] no DNA, only enzyme
374ul [A] ✓
51ul H_2O ✓
425ul

[B] template + primers
374ul [A] ✓
17ul 20uM anchor primer ✓
17ul 20uM M6681 primer ✓
17ul 50 pg/ul M13RF ✓
425ul

use 25ul per 50ul rxn → this will give { 400nM primers, 50 pg template, 50mM KCl, 200 uM dNTPs } in 50ul rxns

up each rxn in duplicate

r components:

	rxn #	1,2	3,4	5,6	7,8	9,10	11,12	13,14	15,16
		small smear condition = rxn buffer	tricine instead of Tris	1.05 mg 2+ instead of 1.5	MgOAc instead of $MgCl_2$	change Mg2+ + anion	broad smear condition	Tricine w/ high Mg^{2+}	Tricine w/ $MgCl_2$
Tris pH 8.4		✓		✓	✓	✓			
KCl		✓	✓	✓	✓	✓	✓	✓	✓
$MgCl_2$		✓	✓						
Tricine pH 9			✓				✓	✓	✓
n $MgCl_2$				✓					✓
MgOAc					✓			✓	
m MgOAc						✓	✓		

To Page No._____

& Understood by me,	Date	Invented by	Date
Debra	7/24/95		7/14/95
		Recorded by Carolyn Combs	

Project No._____

96 Book No._____ TITLE _____

From Page No.__

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
100mM Tris 8.5	10	10			10	10	10	10	10	10							10	10														
100mM Tricine 9			10	10							10	10							10	10												
7.5 ~~15~~ mM MgCl	10	10	10	10													10	10	10	10					10	10	10	10				
5.25 ~~10.5~~ mM MgCl					10	10										10					10	10										
7.5 ~~15~~ mM MgOAc							10	10															10	10								
5.25 ~~10.5~~ mM MgOAc									10	10	10	10													10	10	10	10				
mix	[B] 25ul = template + primers + dNTPs + KCl																[C] 25ul = H_2O + dNTPs + KCl "No DNA"															
0.2 ul Taq (5-7-95 Liq)	5ul brings in ~5% glycerol																															
	add enz, vorter, quick spin (4°C) to start runs																															
	50ul then cycle in Lab15 9600 program 76																															
																	1 min 94°C															
																	30 sec 94°C															
																	30 sec 55°C															
																	2 min 72°C															
																	4°C															
																	4ul															
																	STOP runs w/ 4ul 10X STOP = 10mM EDTA p. 79															
																	8x blue ice															
																	Run 25ul on 1% agarose gel															

T Pag

Witn ssed & Und rstood by m ,	Dat	Inv nt d by	Dat
	7/24/95	R c rd d by Sandra Pombo	7/14/95

g N ._____

→ Tris w/ 1.5mM $MgCl_2$
→ Tricine w/ 1.5mM $MgCl_2$
→ Tris w/ 1.05mM $MgCl_2$
→ Tris w/ 1.5mM MgOAc
→ Tris w/ 1.05mM magnesium acetate
→ Tricine w/ 1.05mM MgOAc
→ Tricine w/ 1.5mM MgOAc
→ Tricine w/ 1.05mM $MgCl_2$

} template + primers

} no DNA ie no template no primers

ecomb 5/14/95 ec

all at 50mM KCl

wer concentration of Mg^{2+} in Cheng vs PCR buffer is responsible for the broader smear. The Tris / tricine pH difference and $MgCl_2$ vs MgOAc do not affect the transition from small (narrow) to broad smear.

is model: distance between primers
low [Mg]
high [KOAc]
low [Tne]

short = small narrow smear

long = broad smear

✓ by decreasing primer annealing
✓ by inhibiting Tne from binding primers

To Page No._____

& Understood by me,	Date	Invented by	Date
clamp	7/21/95		7/14/95
		Recorded by Carolyn Comb	

Page No. _____

15 9600 - 94°C 1min
44°C 30sec
55°C 30sec } 35 cycles
72°C 2min
4°C hold

rxns by adding 7.5 ul of Stop soln = 2x Blue Juice Cf = 1x
100mM EDTA Cf = 12.5mM EDTA

Stop soln made for future use = 50% glycerol
100 mM EDTA (10X)
0.6x TAE
BØB

for 5mL: 2.5ml 100% glycerol
1mL 0.5M EDTA
1.5mL 1x TAE = 30ul 50x TAE stock + 1470 ul H_2O
+ pinch of BØB

To Page No. _____

& Understood by m ,	Date	Invent d by	Date
Polaiyp	7/14/95	Recorded by Cmb	7/15/95

Project No.
100 Book No. TITLE Buffers

From Page No.

Buffer C, 1L ref NB 9, pg 162

17.1 mL 1m K phos monobasic
7.9 mL 1m K phos dibasic
80 mL glycerol
149.12g KCl Cf = 2M
0.2 mL 0.5m EDTA
350 ul 14.3M BME - add
qs to 1L w/ H_2O

1m K phos monobasic
68.045g
qs to 500mL

1m K phos dibasic
114.115g
qs to 500mL

+ 1mL of 50% Tween 20 + NP40 - post filter det 1m/L + B-ME

Buffer D, 8L ref. NB 9 p 182

200mL 1m Tris 7.5
1.6mL 0.5m EDTA
640mL glycerol
2.8mL 14.3 m BME - add
29.8g KCl Cf = 50mM
qs to 8L

make 0.01% NP40 + Tween 20 for dialysis → 2L D + 700ul 400ul 5.0% N
& 0.05% for Heparin column - filter 1L + add 1mL 5.
NP40 + Tween 20 + 350ul.

Buffer C, 500mL ref. NB 9 p 182

det 0.05% final 1mL/L

12.5mL 1m Tris 7.5
0.1mL 0.5m EDTA
40mL glycerol add
0.175mL B-me
74.5g KCl Cf = 2M
qs to 500mL

+ 0.5mL 50% Tween 20 + NP40 after filtering

T Pag N

Witness d & Understood by me, Date 7/24/95 Inv nt d by Dat
R c rd d by 7.18.95

No. 7/18/95

ml Taq extract buffer (P.160, 3) with 0.05% each NP40 / Tween 20
50ul .10mM PMSF
7.6ul 143mM BME
25ul Tween 20 > 50% mix of both → 5mL each
25ul NP40

g cells -70°C Thorton shelf, Lab 16 - chip off 9503-15-764-01-001R
Tne 5 Pol
0 ml Taq extract buffer mix w/ spat in 50ml
10ml 0.2g cells/mL + 10ml pipet Falcon

ake tune - on xL2020 → to tune { tune, turn small to min
then min 1-5 minimize, not over 70
stop

9x 30sec pulses in ice-H_2O bath, ~1min between
pulses - should turn browner

75°C in Falcon - H_2O bath, then cool in ice-H_2O

e Cf 200mM NaCl + 5% PEI to Cf = 0.5% → 16mL vol extract in ~~grad~~ cylinder
58.449/m 30mL centrifuge tube

$.2\ \frac{mole}{L} \times .016\ L = 0.0032\ m$

$\frac{x\ g}{58.449/m} = 0.0032\ m$

0.187g NaCl ✓

~~1.05mL~~ 1.08mL ✓

$\frac{.5\%\ 16mL}{5\%}$ ~~16mL~~ of 5% PEI while extract is
stirring in c tube, 1 drop/sec

15min, 4°C
15min, 15K SS34, 4°C (~2700xg) DNA, cell debris & heat-denatured proteins will ppt.

ant into 25mL grad cylinder:
= FI' fraction 13.25mL of FI' - .2mL = 13.05mL
d AmSO4 to help it go into soln ⇒ 2.297g ammon. sul

To Page No.

d & Understood by me,	Date	Invented by	Date
[signature]	7/24/95	[signature]	7-18-95
		Recorded by Carolyn Combs	

Project No._____

102 Book No._____ TITLE _____

From Page No.___

remove 200ul aliquots of each fraction - eppendorf an
add Am SO4, spin stir 15 min, spin down 15K. 15', remove aliquot remeasure volume

~~S30~~	0% SAT Am SO4	vol (mL)	g Am SO4 slowly, stir 15'
Fr I'	0	13.05	2.297g
S 1	30%	13.8	0.414g ✓
S 2	35	13.5	0.182g wrong 0.223g more added
S 3	~~40~~ 40	~~13.5~~ 13.75	~~0.405g 0.223g~~ 0.426g ✓ aliquot had
S 4	45	13.5	0.419
S 5	50	13.5	0.432
S 6	55	13.4	0.442
S 7	60	13.2	
S 8	65		
S 9	70		

mix: A { 150 ul 0.5 M Tapes 9.3 ×2 = 300ul 0.5 M Taps 9.3
6 ul 1M MgCl2 12 ul 1M MgCl2
50 ul 3M KCl 100 ul 3M KCl

made 2
~~4 mL~~ 6.4 mL ✓

3 mL
206 ul mix A 412 ul mix A ✓
6.3 ul ^{32}P dCTP ~~@~~ 10mCi/mL rcd 7/14/95 - 12.6 ul ^{32}P dCTP α ✓
60 ul 10mM dNTPs 120 ul 10mM dNTPs ✓
405 ul 3.7 mg/mL gap activated DNA 810 ul 3.7 mg/mL activa
2.523 mL H_2O 5.046 mL H_2O ✓
~~3.2 mL~~ for 66 rxns worth, use 48 ul/rxn 6.4 mL

1:100 in Taq dilution buffer 2ul aliquot + 198ul Taq dil bu
5 2ul dil + 48ul reaction mix
10', 74°C 50ul rxn
stop ~1 10ul 0.5M EDTA + 10 ul stop
spot 20ul on GFC filters spot 20ul

T Pag N

Witnessed & Understo d by m , Dat 7/24/95 Inv nted by Dat 7.27.95
R c rd d by

g N .

$\left(\frac{cpm}{specific\ activity}\right)\left(\frac{40\,ul}{20\,ul}\right)(= pmole$

pmol units/ul

1 CPM1
7195 c. combo

1	no cnt	71.00	7.55
2	FI'	4679.00	498
3	s1	5411.00	576
4	s2	5860.00	623
5	s3	5434.00	578
6	s4	3558.00	377
7	s5	1394.00	148
8	s6	299.00	31.8

7.47

→ 55% ammonium sulfate

9	mix	45046.00
10	mix	44957.00
	mix	45141.00

2ul

$\bar{x}$ = 45048

specific activity = $\left(45048\,cpm\right)\left(\frac{50\,ul\,rxn}{2\,ul}\right)$ = ~~##~~ $28.2\ \frac{cpm}{pmole\ nt}$

~~20,000~~ ~~5000~~ (4) pmole nt
10,000
pmole dCTP

$\frac{498\ pmole}{10\ nmole} \times \overset{for\ 10'}{3} = \frac{units\ (200)}{2} = 7.47\ \frac{units}{ul}$ in FI'

100,000 units / 3.5g cells = 28,000 u/g
Liq - 22,000 u/g

To Page N .

ed & Understood by me,	Date	Invented by	Date
Dolores	7/24/97	Recorded by Carlos Combs	7.17.97

PAGE 104 OF NOTEBOOK WAS BLANK

Large scale (31.5 g cells)

Tne prep (continued P176, 9)

N — except use detergent in Tag ext buffer

31.5 g — 9503-15-714 01-001R Tne 5 prep

326 ml — Tag ext buffer (RT) PIPES, 3 : with 0.05% each Tween 20 / NP40

(Cf ~ 0.2 g/ml cells

stir cells in RT buffer in beaker

strain thru cheesecloth

Minigaulin

10,000 PSI / 1 pass

12 mL = FRI Tne, spun down cells 15 min 4°C - sup = FRI Tne

37°C, 15 min (total time after Temp reaches 37°C)

cool / put in ice ~~slurry~~ slurry

vol = 405 ml

NaCl added to Cf 200 mM

= 4.73 g ✓

200 mmole/L × 0.405 L = 81 mmole = 0.081 mole

x g / 58.449/m = .081 m

PEI for Cf = 0.5% add

45 ml 5% PEI pH 7.4 ✓ ~~add~~

add Dropvial, stir 15' more ✓

spin GSA 13,000 RPM / 30 min ✓ Supe = FrI'/PEI

ammonium - divide in 2 / 2 bottles

To Page No.

I & Understood by me,	Date	Invented by	Dat
Steve	7 July 94	Recorded by	7-18-95

From Page No.__

vol of FRI'/PEI sup = 355 mL
removed 1/2 ml = FrI'/PEI

$\frac{35.19}{1000mL} = \frac{12.469}{355mL}$ + ref R. Scopes. Protein Purification p. 304

add 12.469 g ammonium sulfate (ground up) for 55% sat
added slowly over ~ 15 min while stirring at 4°

stirred 15 min, 4°C

spun down pellet 13K, 30 min GSA RC-5B, 4°C → pellet contains
(spin in two bottles to produce 2 pellets of equal size) the DNA polyme
saved sup at 4°C in case activity didn't come down
also 1/2 mL aliquot
respun 5K 5min to pull as much liquid off the pellets as po
put the 2 pellets at -70°C

METHOD 5 BANK 2

0.00 CONC %B 0
0.00 CONC %B 0
0.00 ML/MIN 1.
0.00 PORT SET 6
0.00 PORT SET 6
0.00 VALVE POS 1
VALVE POS 2
CONC %B 0
ML/MIN 1.

FPLC method for S200 column
method 5 bank 2

0	conc % B	0.0
0	conc % B	0.0
0	mL/min	1.5
0	port set	6.1
0	port set	6.1
0	valve pos	1.1
0	valve pos	2.1
400	conc % B	0.0
400	mL/min	1.5

To Pag

Witnessed & Understood by me,	Date	Invented by	Dat
	7/24/95		7-19-95
		Recorded by	

Page No.____ – wash with H_2O, (Hi Trap Pharmacia)
equilibrate a 10 ml blue column (used once and stored in 20% EtOH P191,9)
at 3 col vol/hr = 0.5 ml/min
for 2 1/2 hr (= 7 col vol) with buffer B

wash S200 with H_2O (180 ml = 1 col vol P178,9)
Equilibrate with buffer B
at ½ col vol/hr
= 90 ml/hr
= 1.5 ml/min
} for S200 col will equilibrate O/N at 0.6 ml/min (= 3 col vol) in 500 ml in 14 hr

S200 column

Resuspend one of the two $AmSO_4$ pellets of P.106 (= 0.5 of the total material from 1.5 g cells on P105) in buffer B (~~and~~ containing detergent) (as per P178, 9)
final vol = 0.89 ml spun out insoluble material in microfuge 15'
remove ~20 µl to assay later $AmSO_4$ resuspend

Load by gravity on 180 ml S200.
elute at ½ col vol/hr (ie. 1.5 ml/min) in buffer B
spun = 2A, 2 mm/min, 1.5 ml/min, 3 ml/frn 2 min/frn

To Page No.____

d & Understood by me,	Date	Invented by	Date
D Calvo	7/24/91	Recorded by Carolyn Comb	7-19-91 7-20-91

From Page No.__

unit assay on fractions 3-17 from S200 column
2ul fraction + 61.25ul Taq dil buffer ⇒ 1:31.62
↳ 2ul + 61.25ul Taq dil buffer ⇒ 1:31.62 total of 1:1000 di
↳ 2ul of 1/1000 dil + 48ul mix A p.102
& a no enz control
/ 10min 74°C - temp varied between 72-77°C
/ 10ul STOP 0.5M EDTA
/ 20ul on GFC - wash 1x 10%, 3x 5% TCA, 2x EtOH
/ dry 20min, count

$\frac{\text{pmol}}{\text{unit}} \left(\frac{\text{cpm}}{\text{S.A}}\right)\left(\frac{60\text{ul}}{20\text{ul}}\right)$ specific activity = 28.2 cpm/pmol

SAM		CPM1		unit
1	no enz	41.00	4.36	no enz
Fraction	2 3	52.00	5.63	3
&	3 4	51.00	5.43	4
from	4 5	45.00	4.79	5
S200	5 6	153.00	16.3	6
	6 7	721.00	76.7	7 34.5
	7 8	634.00	67.4	8 30.
	8 9	2027.00	216	9 97.2
	9 10	4597.00	489	10 22C
	10 11	301.00	32	11 4
	11 12	344.00	36.6	12
	12 13	820.00	87.2	13
	13 14	208.00	22.1	14
	14 15	531.00	56.4	15
	15 16	321.00	34.1	16
	16 17	254.00	27.0	17

3/24/95 c

pool fr 9, 10 = 6 ml total
↳ = 317,250 units loaded onto Blue Sepharose

unit def:
unit/ul → inc. 10,000 pmole at 30min ⇒ ~~2027~~ $\frac{216\text{ pmol}}{2\text{ul}} = \frac{108\text{ pmol}}{\text{ul}} \times 1000 \times$ c

$= 1.08$

$1.08 \times 10^5 \frac{\text{pmol}}{\text{ul}} \left(\frac{30\text{min}}{10\text{min}}\right) = 3.24 \times 10^5 \frac{\text{pmole}}{\text{ul}}$

$\frac{10{,}000\ \text{pmole}}{\text{unit}}$

$= 32.4\text{ u/ul} \times 300$

$= 97{,}200\text{ units}$

T Page

Witness d & Und rst d by m ,	Date	Inv nted by	Dat
		R cord d by	

g 'N

Cut sepharose

Wash for 9.10 of S200 (P10?) V_f = 6 ml

1 col vol/hr = 10 ml/hr = 0.17 ml/min

Wash O/N

7 col vol buffer B total = 70 ml

(0.08 ml/min ≈ 70 ml in 15 hr)

collect 10 × 7 ml fractions of wash

77 min/fr

HOD 5 BANK 2

.00	CONC %B	0.0
.00	CONC %B	0.0
.00	NL/MIN	0.08
.00	PORT.SET	6.1
.00	PORT.SET	6.1
.00	VALVE.POS	1.1
.00	VALVE.POS	2.1
.00	CONC %B	0.0
.00	NL/MIN	0.08

FAIL. M=5 B=2
11.31 RE= 11.31

gradient: will scale down gradient for TPI of P182, 9 à

for 10 ml Blue col

200 ml total gradient (20 col vol)

of 50mM - 1M KCl

3 col vol/hr = 0.17 ml/min

3 ml/fr

6 min/fr

1 mM/min

C Combs
7/21/95

start ~ 7.45 AM

To Page No.

d & Understood by me,	Date	Invented by	Date
		Record by Carolyn Combs	7-20-95 7-21-95

g No.__

Cut sepharose

Load for 9.10 of S200 (P10F) V_f = 6 ml

1 col vol/hr = 10 ml/hr = 0.17 ml/min

Wash O/N

7 col vol buffer B total = 70 ml

(0.08 ml/min = 70 ml in 15 hr)

collect 10 × 7 ml fractions of wash

77 min/fr

HOD 5 BANK 2

.00	CONC %B	0.0
.00	CONC %B	0.0
.00	ML/MIN	0.08
.00	PORT.SET	6.1
.00	PORT.SET	6.1
.00	VALVE:POS	1.1
.00	VALVE.POS	2.1
.00	CONC %B	0.0
.00	ML/MIN	0.08

FAIL. M=5 B=2
11.31 RE= 11.31

gradient : will scale down gradient for TFI of P 1822, 9 ä

for 10 ml Blue col

200 ml total gradient (20 col vol)

of 50mM - 1M KCl

3 col vol/hr = 0.17 ml/min

3 ml/fr

6 min/fr

1 mm/min

C Combs
7/21/95

start ~ 7:45 AM

To Page No.____

& Understood by me,	Date	Invented by	Date
		Recorded by Carolyn Combs	7-20-95 7-21-95

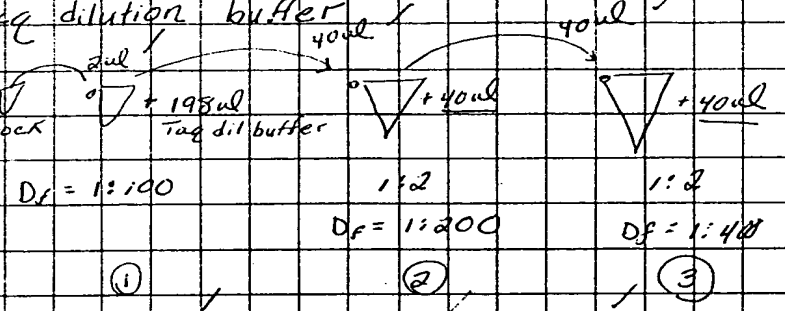
Project No._____

110 Book No._____ TITLE _____

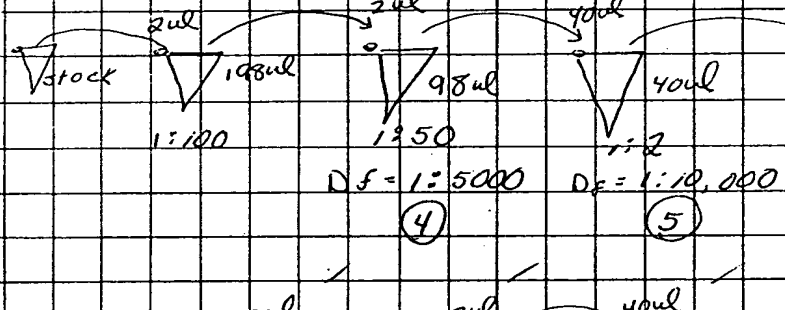
From Pag No.__ 7/21 Unit assay ^{32}P α dCTP ref 7/14/95

dilutions in Taq dilution buffer

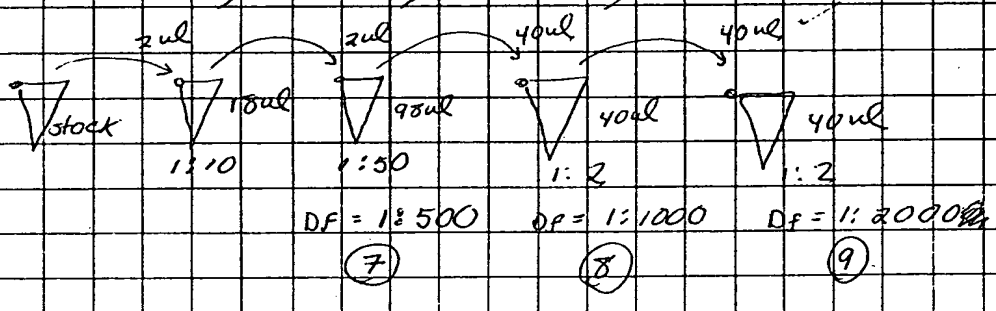
- Fr T1/PET 355mL



- Am SO4 pellet 0.89mL resuspension



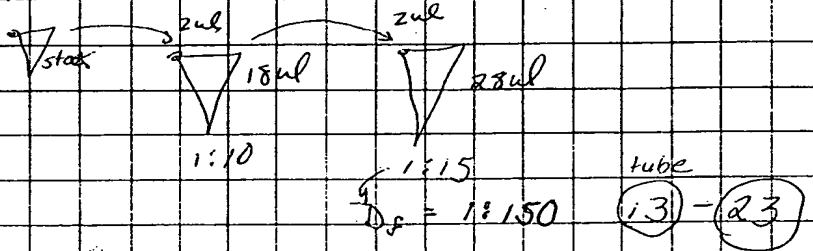
- S200 pooled peak fractions 6mL



- Fractions from Blue Sepharose columns

undiluted wash fractions W5 (10)
W10 (11)
F5 (12)

peak elution fractions 10-20



Start rxns by adding 2ul of each dilution to 48ul A mix p. 102
74°C 1 amin
+10ul 0.5m CDTA, spot & wash ~~spot~~ 20ul

T Pag N

Witnessed & Understo d by m ,	Date	Invented by	Dat
[signature]	7/24/95	R c rd d by Pauline Ponts	7/21/95

e N .

pmole
cpm
specific activity

	CPM1
1100	4504.00
1200	3334.00
1400	1539.00
1000	11945.00
0.000	8088.00
.0000	4497.00
500	9437.00
1000	5329.00
20000	3011.00
ash 5	108.00
ash 16	98.00
r 5	107.00
10	651.00
11	813.00
12	1642.00
13	3668.00
14	7866.00
15	10,929.00
16	6668.00
17	6788.00
18	5668.00
19	3724.00
20	2935.00

frx 13+17

2 ml (of 3 ml) from each fraction V_f = 10 ml

analysis of tRNA prot

Dialyze against 1000 ml buffer D (pH 8.0)

continued on p. 112

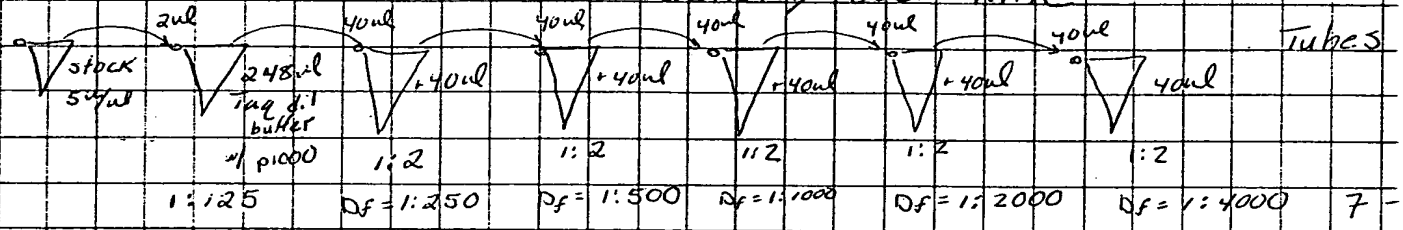
To Page No.

I & Understood by me,	Date	Invented by	Date
	7/21/77	Recorded by Carolyn Combs	7-21-77

From Page No._ Unit assay on old Tne prep (5-7-95) side-by-side the new Tne pool which was eluted from Blue ~~agarose~~ Sepharos column on 7/21/95 p.III NB II.

enzyme dilutions in Taq dilution buffer:

Tne (5-7-95 Liz) - ~~tube~~ previously determined to be 5 u/ul, but i have lost activity over time



same dilutions also made w/ Tne from Blue Sepharose column pool

start rxns w/ 2ul of each dilution into 48ul mix A ref 0.102
10' 74°C, stop w/ 10ul 0.5M EDTA, spot 20ul
^{32}P αdCTP ref 7/14/95

	SAM	CPM1	pmol	u/ul	
Tne (5-7-95 Liz)	1 1/125	2730.00	352	5.44	6.41
	2 1/250	1638.00	290		7.60
	3 1/500	790.00	202		7.00
	4 1/1000	388.00	95		
	5 1/2000	232.00			
	6 1/4000	143.00			
Tne 7/14/95 Sepharose	7 1/125	9450.00			
	8 1/250	5021.00			
	9 1/500	2872.00			
	10 1/1000	1669.00	207	31.0	32.5 ave
	11 1/2000	899.00	107	32	
	12 1/4000	503.00	56	33.8	
	13 no enz	67.00			

∴ Comb 7/22/95

ave ~ 7.0 u/ul

(= 193600 units total in blue pool fr 15-17)

23.2
222 cpm/pmol
as of 7-22-95

T Pag

Witn ss d & Understo d by m ,	Dat	Inv nt d by	Date
	7/24/95	R cord d by	7-22-95

PAGE 113 OF NOTEBOOK WAS BLANK

From Page No.

check conductivity after dialysis of P111 10µl / 1ml H

buffer D	88µS
Hep col effluent	84µS
Dialysate	94µS

∴ conductivity is good and is similar to P183, 9 for TP1

1. Load on ~22 ml Hep equilibrated op with buffer D (P100

at 0.67 ml/min (= 40ml/hr = 2 col vol/h

2. wash 1 col vol

Gradient (start ~9:30AM)

50mM – 1.05M KCl (0.67ml/

using buffer D and E (2M KCl)

∴ 0 – 50% E

600 ml total gradient vol (~30 col vol)

∴ 50 – 700 mM KCl is in 20 col vol same a P185, 9

0.5mm/min span = 2A

To Pag No

Witness d & Understo d by m,	Dat	Inv nt d by	Dat
Polan	7/24/95	Record d by	7.22.95

Storage buffer

Page No.

2L buffer F (as per 91342.PRP)

2 Tris pH 8	40 ml	✓
5 M EDTA	0.4 ml	✓
M DTT	2 ml	✓
0% NP40 Pierce	2	✓
% Tween 2 Pierce	2	✓
Glycerol	1 L	✓
H_2O		
	2 L	

Dialyze vs 1 L for 5 hr. Change to another L for 0/

ut assay on fractions eluted from Heparin column:
do 1 dilution (1/150) of fractions # 35-53 and a series of dilutions on the fraction with the maximum UV absorbance - #44

dilutions in Taq dilution buffer

stock → 2ul → 298ul Taq dilution buffer P1000

1:150 for fractions 35-53, called H35-H53

and ~~1:125~~, 1:250, 1:500, 1:1000, 1:2000, 1:4000 of H44 & 1:8000 - see dilutions on p 112.

do make 1:125 but don't do rxn w/ it

To Page No.

ed & Understood by me,	Date	Invented by	Date
Polcari	7/24/95	Recorded by	7-27-95

Project No._____

Book No._____ TITLE _____

From Page No.__

pmol u/µl

fr 44

1	1:250	2180.00	274	12.3
2	1:500	938.00	114	7.5
3	1:1000	560.00	55	5.8
4	1:2000	398.00	44	
5	1:4000	252.00		
6	1:8000	146.00		

ave 9.53 u/µl

specific activi
= 19.1 cpm/

Hep frac 1:150 dilution 35-53

no Enz	7	57.00			
fr 35	8	1024.00			
36	9	699.00			
37	10	1128.00			
38	11	1127.00			
~~39~~	12	2104.00			
40	13	2484.00	pmole		
41	14	2621.00	412	—	9.27
42	15	4760.00	748	—	16.82
43	16	3657.00	574	—	12.92
44	17	3507.00	550	—	12.39
45	18	4717.00	740	—	16.7
46	19	4120.00	647	—	14.6
47	20	3517.00	552	—	12.4
48	21	3280.00	515	—	11.6
49	22	2282.00			
50	23	1820.00			
51	24	1301.00			
52	25	750.00			
53	26	513.00			

$\frac{412 \text{ pmole}}{2 \text{ ml}} \times 150 \times \frac{30}{10} = 9.27 \text{ u/ul}$

$\bar{x} = 13.3 \text{ u/ul} \pm 2.6 \text{ u/ul}$

pool 41-47

(7 frns) (1.34 ml/frn)

= 10.72 ml total

pooled frn 41-47
(= 10.7 ml total Vol)

Dialyze into buffer E

see p 115

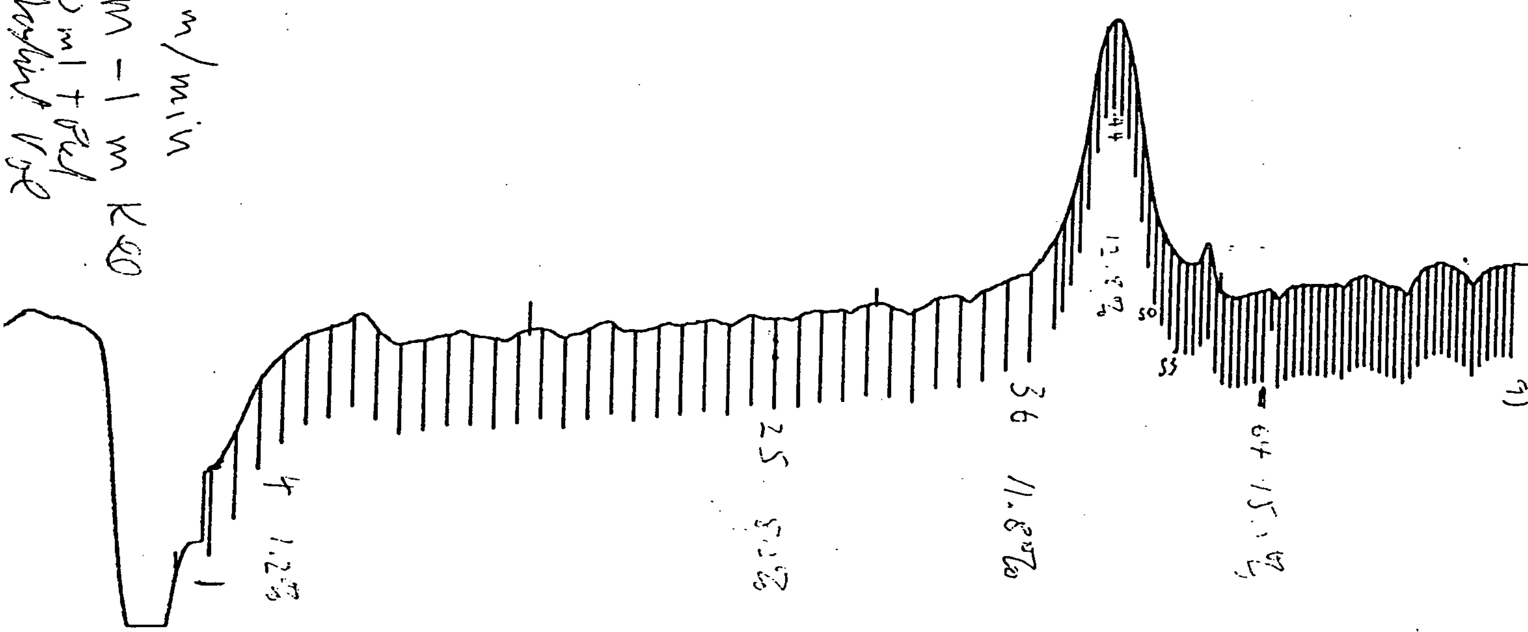
Recovered 2.6 ml The after dialysis (in by
add 2.6 ml buffer G of 4-29-55 (see p 6, 10 and 9134[illegible]

T Pag No

Witnessed & Understood by m,	Dat	Invented by	Dat
[signature]	7/24/55	[signature]	7-22-55
		Recorded by	

24 0.67 ml/min
7-22-95 20ml Heparin TNE
7-22-95 Load
0.5 mm/min
50mM - 1 M KCl
600 ml total
Gradient Vol

2 min/frac



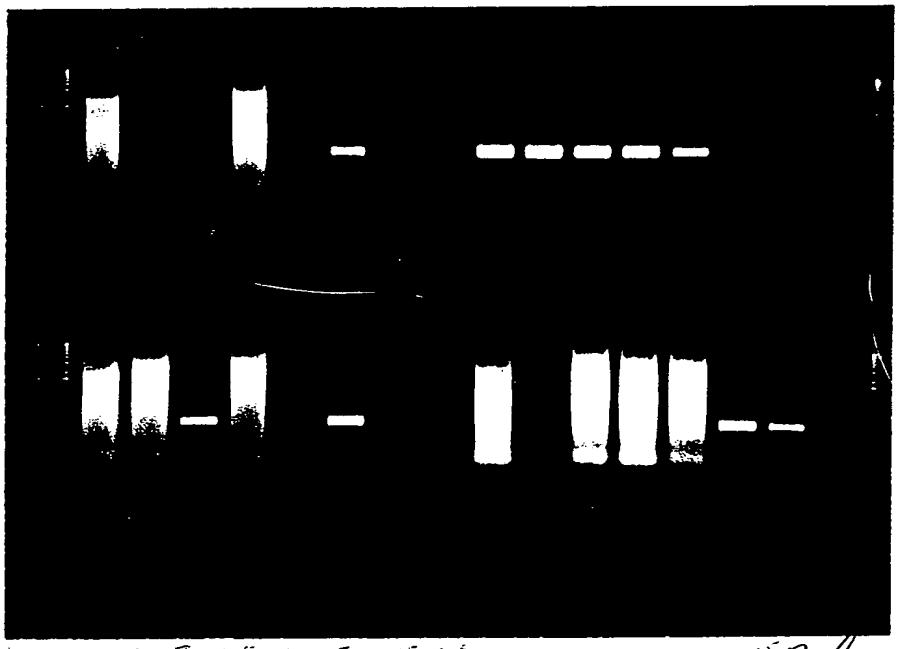
To Page No.______

ed & Understood by me,	Date	Inv nted by	Date
Polaris	7/24/95	Recorded by	7-22-95

This experiment is detailed on p. 119-120, NB 11

The Taq
J-7-95 Liz

50ul rxns units 3 2.5 2 1.5 1 0.5 0.25 0.125



.. 3 2.5 2 1.5 1 .5 .25 .125

7-27-95

Blue Heparin
Seph

Witnessed & Und rst d by me,	Dat	Inv nted by	Dat
	7/24/95	R cord d by	7-27-95

PCR rxns with Blue Sepharose and Heparin fractions of Tne prep 7/22/95

g N — 7/23/95

tion of Blue Sepharose pool fractions and Heparin pool fraction of Tne. aq and Tne (5-7-95) prep will be tested in PCR alongside the new Tne. n we use more than 2 units of new Tne and not get a smear? e 5-7-95 Tne prep gives a smear w/ more than 1 unit (p. 83 NB11)

ions:
heng buffer
5 cycles w/ old program ie lab 15 9600 #76 94°C 1 min
st 0.125, 0.25, 0.5, 1, 1.5, 2, 2.5, 3 units 50ul rxn 35x { 94°C 30sec; 55°C 30sec; 72°C 2 min } 4°C
ke the 380 bp product

cktail w/ all components except enzyme, for 34 rxns

340 ul	5x Cheng	
34 ul	10 mM dNTPs	
34 ul	50 pg/ul M13 RF	
34 ul	20 uM anchor primer	> see p. 42 NB11
34 ul	20 uM 6681 primer	
1156 ul	H_2O house distilled	
1632 ul	→ 48 ul / PCR tube for 9600	

zyme dilutions in Taq SB:

r Tne (5-7-95 liq) and rTaq - both 5u/ul do the following dilutions

stock 5u/ul	+23.3 ul Taq SB (10 ul)	+4 ul (20 ul)	+5 ul (20 ul)	+6.6 ul (20 ul)	+10 ul (20 ul)	+10 ul (10 ul)	+10 ul (10 ul)	+10 ul (10 ul)
Df =	1.5 u/ul	1.25 u/ul	1 u/ul	0.75 u/ul	0.5 u/ul	0.25 u/ul	0.125 u/ul	0.0625 u/ul

start rxns w/ 2 ul of each dilution on ice, flick, spin down

r Blue sepharose pool - 23.1 u/ul when normalized to 5-7-95 Tne
ie. $\frac{5}{7}(32.3) = 23.1$ p. 112, NB11

stock 23.1 u/ul → (10 ul) 36.2 ul Taq SB 1:4.62 5 u/ul

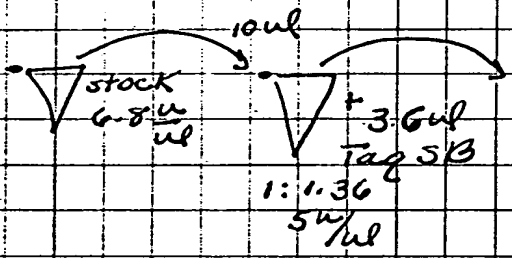
same as Tne (5-7-95) dilutions

To Page No.

ed & Und rst od by me,	Date	Invented by	Date
Polanyi	8/1/95	Recorded by Carolyn Comb	7/27/95

From Page N

Heparin fraction #44, which had peak UV absorbance is 6.8 u/ul when normalized to Tne (5-7-95 prep) i.e. $\frac{5}{7}(9.53) = 6.8$ u/ul p. 116 NB II



same dilutions as for Tne (5-7-95) p

start rxns w/ 2ul of each dilution starting w/ the 1.5 u/ul dilutions

35 cycles, stop w/ EDTA stop soln, 7.5ul
run 25ul on gel

Result on p. 118 NB II - new Tne prep (7-22-95) is not less prone to making a smear than the old (5-7-95) prep. So, DNA contamination of enz should not be Tne's main problem. Rather, Tne may have an intrinsic activity that makes it "smear" more easily than Taq

T Pag I

Witness d & Und rstood by me,	Dat	Inv nt d by	Dat
	8/1/95	R corded by	7/27/95

Project No._____
Book No._____ TITLE _____

Exhibit 140
Appl. No. 09/558,421

Fr m Page No.__

1) Can rTaq make a smear in high Mg^{2+} ? ± primers ± temp

mix A = ~~494.4~~ ~~510.4~~ ul H_2O — for 8 rxns — 8 units

80 ul 10x PCR buffer ✓
~~16 ul~~ 10mM dNTPs ✓
16 ul 50 pg/ul M13 RF target ✓
16 ul 20uM anchor ✓
16 ul 6681 primer, 20uM ✓
~~1.31~~ ~~1.6~~ ul rTaq ~~5 u/ul~~ 8.28 u/ul
.29 ul H_2O ✓
640 ul

mix B = 542.4 ul H_2O — for 8 rxns

80 ul 10x PCR b
16 ul 10mM dNTP
~~1.31~~ ~~1.6~~ ul rTaq 5 u/
.29 ul H_2O ✓
640 ul ✓

start rxns by adding Mg^{2+}

#	1 (underlined)	2	3	4	5	6	7	8	9	10	11	12	13
H_2O	17.9	17	16	12	8	4	0	17.9	17	16	12	8	4

mix A ✓ = + primers template — 80 ul

mix B ✓ no primers no template — 80 ul

add last just before PCR

50mM $MgCl_2$	2.1	3	4	8	12	16	20	2.1	3	4	8	12	16

100ul rxns

35 cycles
stop whole rxn w/ 11 ul stop soln w/ 10x EDTA p 79 ✓

To Pag N

Witnessed & Understood by me,	Date	Inv nt d by	Dat
	8/1/95	Record d by Pamela Pomb	7/27/95

N

xes Tne work better (make more product) with a hot start?

mix C for 4.5 rxns = 90 ul 5x Cheng ✓
301.5 ul H_2O ✓
9 ul 50 pg/ul m13 RF in TC 50 from 2/94 ✓
9 ul 20 uM anchor primer ✓
9 ul 20 uM 6681 primer ✓
9 ul 10mM dNTPs ✓
427.5

R Tne 7/22/95
real 19.0 u/ul
1:30.5x dilution
6 : 183
6 ul new Tne
177 ul Taq SB

95 ul + 5 ul Tne 0.2 u/ul hot start (15)

duplicate hot start (16)

cold start duplicates (17) (18)

stop at 25, 30, 35 cycles
20 ul + 3 ul stop

added enz after 1' 94°C denaturation → HSX 2

Can human spleen genomic DNA promote smear formation - bad seed?
no template, no primers

high Mg^{2+} - short smear condition
2.5 rxns

25 ul 10x PCR buffer ✓
187.5 ul H_2O ✓
5 ul 10mM dNTPs ✓
7.5 ul 50mM $MgCl_2$ ✓
225 ul

mix E

low Mg^{2+} - long smear condition

25 ul 10x PCR buffer ✓
189.75 ul H_2O ✓
5 ul 10mM dNTPs ✓
5.25 ul 50mM $MgCl_2$ ✓
225 ul

90 ul D ✓
5 ul H_2O ✓
5 ul genomic D.NA 80 ng/ul TC/S ✓
5 ul Tne, 0.2 u/ul (19)

90 ul D ✓
5 ul H_2O ✓
5 ul Tne (20)

90 ul E ✓
2.5 ul H_2O ✓
2.5 ul genomic DNA ✓
5 ul Tne (21)

90 ul E ✓
5 ul H_2O ✓
5 ul Tne (22)

remove 10 ul at 15, 20, 25, 30, 35 cycles STOP tubes + 2 ul stop

To Page No.

d & Understood by me,
Solange

Date 8/1/95

Invented by

Recorded by Evelyn Combs

Date 7/27/95

Project No.

124 Book No. TITLE

From Page No.

4) Does Tne make a smear when 1 or 2 dNTPs are missin from rxn?

4 dNTP mix, 2.5mM each ⇒ 20ul each 10mM dNTP stock
T ✓ C ✓ G ✓ A ✓

3 dNTP mix, AGC 2.5mM each ⇒ 20ul 10mM A ✓
20ul 10mM G ✓
20ul 10mM C ✓
20ul H_2O ✓

2 dNTP mix, GT 2.5mM each ⇒ 20ul 10mM G ✓
20ul 10mM T ✓
40ul H_2O ✓

} use 8ul of mixes in each 100 PCR rxn for C_F = 2

mix F (for 20 rxns) = 200ul 10x PCR buffer ✓
1480ul H_2O ✓
1680ul

G (for 9.5 rxns)
798ul ✓
28.5ul 50mM $MgCl_2$ ✓
883.5ul

H
798ul ✓
19.95ul 50mM M[illegible] ✓
8.55ul H_2O ✓
883.5ul

J: 261ul G, 24ul 4 dNTP ✓
K: 261ul G, 24ul 3 dNTP (= 3 dNTP mix) ✓
261ul, 24ul 2 dNTP ✓

L ✓ M ✓ N ✓

95ul
+5ul Tne .24 U

23, 24 | 25, 26 | 27, 28 | 29, 30 | 31, 32 | 33, 3[illegible]

100ul

35 cycles
11ul stop (p.79)

Lab 16, 9600 method 103 1:25pm

T Pag N

Witnessed & Understood by me, [signature]
Dat 8/1/95

Invented by [signature]
Recorded by [signature]
Dat 7/27/95

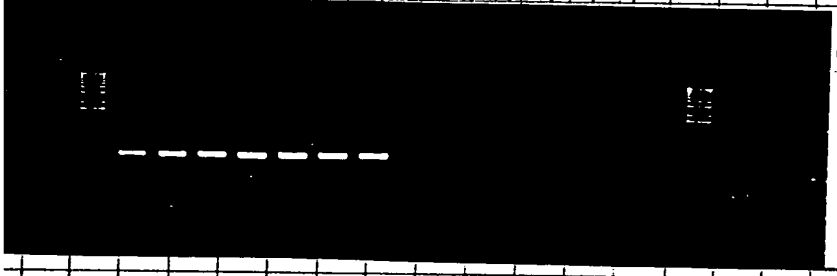
ge No.__

1% agarose gel ⇒ 3.6g agarose
300mL 1X TAE
20ul EtBr 5989

rTaq

target + primers | no input DNA

$MgCl_2$	1.0	1.5	2	4	6	8	10	1.0	1.5	2	4	6	8	10



L. Comb 7/25/95

top
bottom

1 - 14 ⇒ 25ul
15 - 18 12 tubes 20ul inc. cycles
20 tubes 19 - 22 ⇒ 10ul inc cycles ie 19 15C 19 20C 19 25C ect
23 - 34 ⇒ 25ul

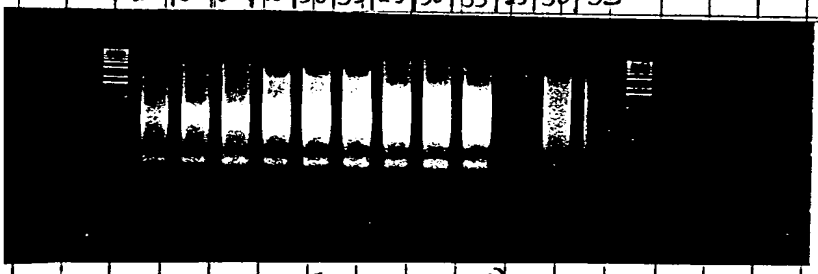
Results

- Increasing Mg^{2+} did not cause rTaq to make a smear either in the presence or absence of input DNA

Tne (7-22-95 prep) ⟶ Cheng buffer primers + target 3ul/50ul rxn

hot start | cold start

cles	25	30	35	25	30	35	25	30	35	25	30	35



L. Comb 7/28/95

- With 3units Tne, the hot start rxns did not smear any less than cold start rxn. I should have used 1.36 units in order to get product instead of smear

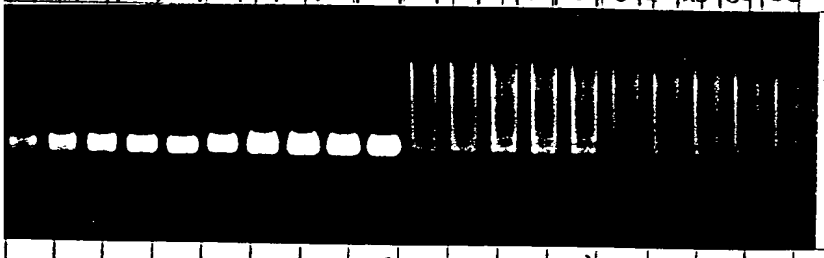
redo this expt w/ 1.36units Tne/rxn

Tne (7-22-95)

1.5mm $MgCl_2$ | 1.05mm $MgCl_2$

PCR buffer 3ul/rxn ⇒ too much

+ genomic	15	20	25	30	35	− genomic	15	20	25	30	35	+ genomic	15	20	25	30	35	− genomic	15	20	25	30	35



L. Comb 7/28/95

- Addition of genomic DNA did not result in more smear over time w/ 3units Tne

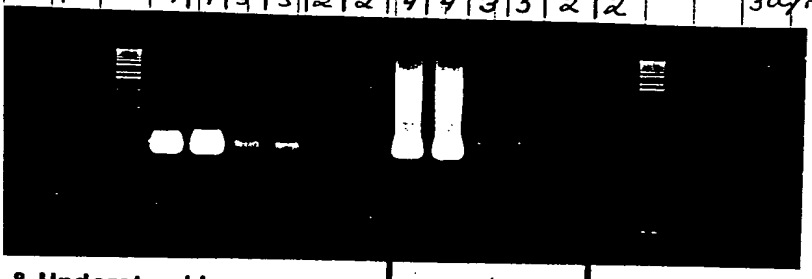
redo this expt w/ 1.36units Tne/rxn

Tne (7-22-95)

1.5mm $MgCl_2$ | 1.05mm $MgCl_2$ → PCR buffer 35 cycles 3ul/rxn

esent	4	4	3	3	2	2	4	4	3	3	2	2

3 dNTP mix = AGC present
2 dNTP mix = GT present



L. Comb 7/28/95

- No smear made when 2 dNTPs (GC or A) are missing, so smear is probably not made by a TdT activity.

To Page No.____

& Understood by me,
Polanyo

Date 8/1/95

Invented by

Recorded by Carolyn Comb

Date 7/27/95

Project No._____

126 Book No._____ TITLE _____

From Page No.__

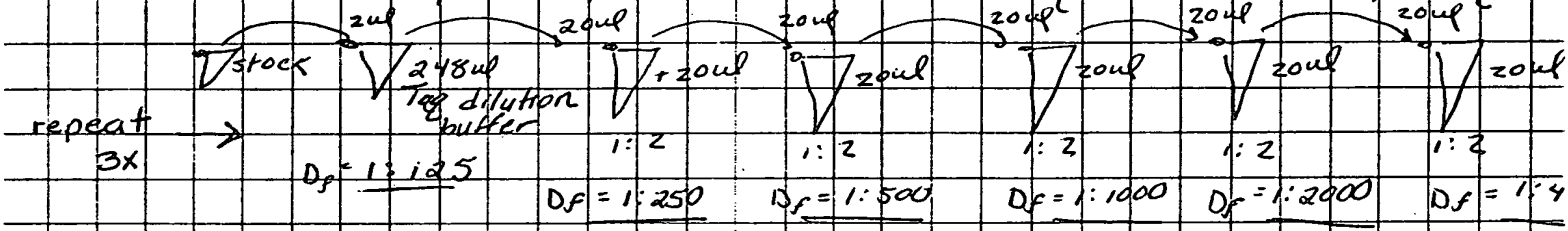
7/26/95 Unit assay of new Tne prep (7-22-95) after dialysis into Taq storage buffer.

- old 5-7-95 Tne prep & rTaq will be done too
- 3 replicates of each dilution series, done 3x independ

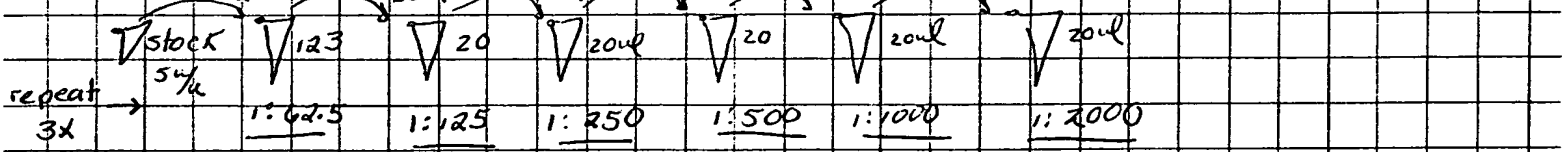
cocktail: 206 ul of Taps, $MgCl_2$, KCl mix –
2,520 ul H_2O w/ p1000
60 ul 10mm dNTPs
405 ul 3.7 mg/mL gapped DNA
9.3 ul ^{32}P αdCTP ref date 7/14/95
3.2mL

use 48 ul per rxn
start rxns by adding 2ul of enzyme dilutions on ice.

Dilutions of ^Heparin pooled fraction ~~rTaq 5B~~ final dilute w/ Taq di



Dilutions of Tne (Liz 5-7-95) and rTaq both labeled 5 u/ul



- rxns stopped w/ 10ul 0.5M EDTA
- 20ul of each rxn spotted on GFC filters →

T Pag N

Witn ssed & Understood by m ,	Dat	Invented by	Dat
	8/1/95	R cord d by Pamela Combs	7/27/95

E. Combs 7/27/95

	SAM	CPM1	ave pmol	ave u/µl	
	1	8081.00			
	2	7346.00	625	11.7	
	3	5871.00			
	4	5139.00			
	5	5341.00	441	16.5	
	6	4559.00			
	7	3009.00			Tue 7-22-95
	8	2963.00	255	19.1	19.0 u/λ ave
	9	2724.00			
1/1000	10	1492.00			
	11	1359.00	125	18.8	
	12	1429.00			
1/2000	13	891.00			
	14	899.00	79		
	15	895.00			
1/4000	16	490.00			
	17	402.00			
	18	524.00			
1/62.5	19	6160.00			
	20	6476.00	582	5.4	
	21	7195.00			
1/125	22	4215.00			
	23	4266.00	354	6.6	
	24	3596.00			
1/250	7	2124.00			Tue Liz
	8	2014.00	181	6.8	6.8 u/λ ave
	9	2055.00			
1/500	10	1160.00			
	11	998.00	95	7.0	
	12	1024.00			
1/1000	13	572.00			
	14	610.00			
	15	609.00			
1/2000	16	361.00			
	17	352.00			
	18	348.00			
1/62.5	19	8453.00			
	20	6925.00	658	6.17	
	21	7075.00			
1/125	22	4769.00			
	23	3803.00	387	7.25	
	24	4613.00			
1/250	25	2896.00			Tue
	26	2565.00	240	9.00	ave 8.28
	27	2722.00			
1/500	28	1185.00			
	29	1404.00	115	8.60	
	30	1334.00			
1/1000	31	1234.00			
	32	873.00	89.7	13.40	
	33	953.00			
1/2000	34	592.00			
	35	527.00			
	36	509.00			
	37	56483.00			
	38	57656.00	56855 ave	= 34.1 cpm/pmol	
	39	56427.00			

expected ~5 u/λ

expected 5 u/λ on ~90 µl/λ real units in nmol/50'

⇒ use (x ul)(19.0) = 1.36 u/100 ul
⇒ 0.0716 ul/100ul PCR rxn

⇒ we had been using 0.2 ul/100ul rxn
0.2 ul is really 1.36 units, not 1 unit as we thought based on the unit value 5 u/ul

⇒ use (x ul)(8.28 u/ul) = 1.36 u/100ul PCR
= 0.164 ul/100ul Taq

To Page No._____

Understood by me,	Date	Invented by	Date
Bolanp	8/1/95	Recorded by Carolyn Combs	7/27/95

Project No._____
Book No._____ TITLE dU containing templates (Mispair Goodman assay)

From Page No.____

"7-11-95 "Fidel pri" (27mer)

Exhibit 141
Appl. No. 09/558,421

GAGACATGGCGTCCCAGTCACGACCT
CTCTGTACCGCAGGGTCAGTGCTGGACTAGTACGAGCTACT
U

27 bp

"Fidel-Temp
or "Fidel-Temp
(7-11-95)
(42-mer

#51351 → This is not old "Fidel T
of pJ1,10. This new ou
less hairpin or primer
according to oligo prop

. ssDNA region is same as MG JBC Creighton & MG F
for + dGTP + dATP (get G-A mismatch at position 3 and)
+ dCTP for rescue

. For test of dATP incorp opposite Template dU,
have all 4 dNTPs present at 200 μM each
and look for pause one site before dU
(run on 20% PAGE?)

```
      G
      C
      A ↑
  U   T A
      G C (31)
      A T
      T A
      C G
primer (27)
```

To Pag No

Witnessed & Understood by me,	Date	Invented by	Dat
Deeraen Bolup	8/1/95	Recorded by	7-28-95

Project No.____
Book No.____ TITLE dU vs dT in template

From Page No.__

	①	②	③	④	⑤	⑥	⑦	⑧
purified Temp dT / 50 nM primer (P55)	4 →			→				
purified Temp dU / 250 nM primer (P55)					4 →			→
10X Taq PCR buffer	10	10			10	10		
50 mM MgCl	3	3			3	3		
10X Vent buffer			10	10			10	10
10 mM dNTPs	2 µl →							→
rTaq 0.0625 u/µl	2				2			
Tne 0.0625 u/µl		2				2		
Vent 0.0625 u/µl			2				2	
DeepVent 0.0625				2				2
H_2O	79 →		82 →		79 →		82 →	

100 µl

preheat to 70°C
Start with addition of pol.

remove 10 µl to 5 µl cycle seq stops at
0 5 10 20 40 60 90

10 nM / ~1 pm total / 1.5 m / C / 20 / dNT / .125 / 2 to / 0.013 pm pol m / ~ prime / pol / so huge primer overpo

T Page No

Witnessed & Understood by me, [signature] Date 8/1/95
Invented by [signature] Date 7-28-95
Recorded by

ge N .

sequence Rxn same as P 274 and 9011
casing 32P prefield Temp with dT and dA

To Page No.

ed & Understood by me,

Dat 8/11/95

Invented by

Recorded by

Date 7-28-95

Project No.

128 Book No. TITLE Effect of annealing temperature on Tne smear

From Pag No.

- anneal at 50°C, 60°C, 70°C w/ 72°C extension - no input DNA
- take out rxn aliquots after 5, 10, 15, 20, 25, 30, 35 cycles
  → use 1.36 units Tne (5-7-95 Liz) / 100ul rxn
- test the effect of annealing temp on both the long (low Mg^{2+}) and short (hi Mg^{2+}) smears

mix A = high Mg^{2+} for small smear - enough for 3.5 rxns

280 ul H_2O
35 ul 10x PCR buffer ✓
7 ul 10mM dNTPs C_f = 200uM ✓
10.5 ul 50mM $MgCl_2$ C_f = 1.5mM ✓
332.5 ul

mix B = low Mg^{2+} for long smear - enough for 3.5 rxn

283.15 ul H_2O
35 ul 10x PCR buffer ✓
7 ul 10mM dNTPs
7.35 ul 50mM $MgCl_2$ ✓ C_f = 1.05 mM
332.5 ul

* 25 fold dilution
Tne stock (Liz)
5 u/ul (real units p.127
3ul Tne
72ul Taq
75 ul of 0.

annealing temp	50°C Lab 15		60°C Lab 16		72°C / 70°C Lab 562		
rxn #	1	2	3	4	5	6	← 5 & 6 received mix that were made up a different time th the mix A & B for 1
1 ul Mix A hi mg	95		95		95		
1 ul Mix B low mg		95		95		95	
* ul Tne 0.2 u/ul (5-7-95 Liz)	5	→	→	→	→	→	

100ul rxns

remove 10ul of rxn to 2ul STOP soln w/ EDTA p. 79 NB 11
at cycles 10, 15, 20, 25, 30, 35

To Pag

Witness d & Understo d by me,	Dat	Inv nted by	Dat
[illegible]	8/1/95	R cord d by [illegible]	7/28/95

g N .

:ling Lab 15 program 76 94° 1min
35x { 94° 30sec / 50° 30sec / 72° 2min }
4°C —

Lab 16 program 103 94° 1min
4.5 min per cycle
35x { 94° 30sec / 60° 30sec / 72° 2min }
4° —

SG1 program 133 94° 1min
~~72° 2min 30sec~~
2 temp. PCR 35x { 94° 30sec / 72° 2min 30sec }
4° —

2% agarose gel, 36 samples + primers [2ul zoumanchor, 2ul zoum 6681, 96ul H_2O, 11ul stop, load 20ul] + uncycled Tne [1.5ul Tne Liz, 36ul SIG, 45ul 95ul H_2O, 11ul stop, load 20ul]

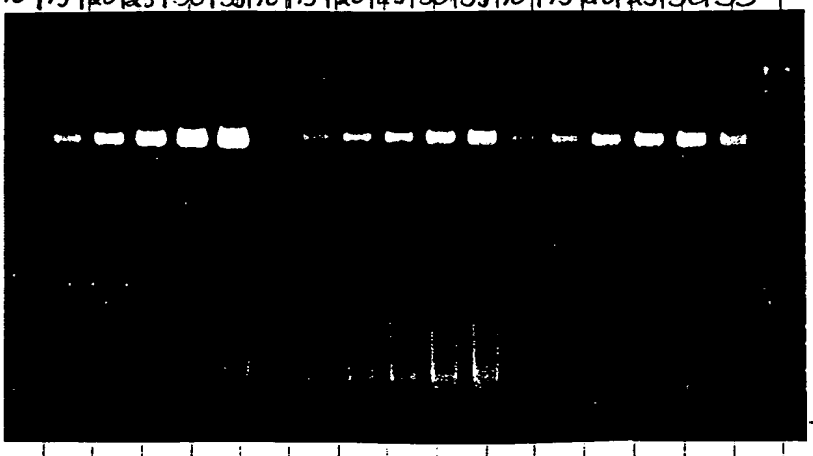
2
1/3 { short 50° 10 to 1-35 ; 3-10 to 3-35 ; 5-10 to 5-35 }
1/3 { long 50° 2-10 to 2-35 ; 4-10 to 4-35 ; 6-10 to 6-35 }
1/3 { 60° 10 to 3-35 ; 10 to 4-35 ; 10 to 6-35 }
1/3 { 72° 10 to 5-35 ; 10 to 6-35 }

primers, 7/5/95 Tne, 7/22/95 Tne

Tne (5-7-95 Liz) 1.36 u/rxn

ng

50°C						60°C						72°C					
10	15	20	25	30	35	10	15	20	25	30	35	10	15	20	25	30	35



1.5mM $MgCl_2$

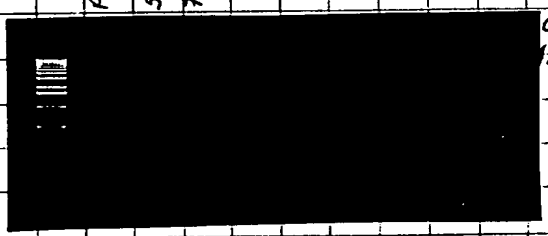
1.05mM $MgCl_2$

7/28/95

primers alone, 5n moles and 8n moles 6681

5-7-95 Tne

7-22-95 uncycled



Comb 7/28/95

To Page No.

sed & Understood by me,	Date	Invented by	Date
	8/1/95	Recorded by Carolyn Combs	7/28/95

Project No._____
Book No._____

TITLE same as P121,9 and P52,10 — unit assay for 1.1X Taq

From Pag No.__

		Rxn#	ul/assay	1% Tween20/NP40 ea
4°C #10 (P121,9) (no det)		1-3	2	1 µl
11	1.1X	4-6	3.64	
1	(2X 0.1% T/N)	7-9	2	
14	(2X Tfl buffer)	10-12	2	
nTaq (same as P121,9) 1/25 dil		13-17	2	
~~Fidel Fort~~ 1.1X called "new" on P54 (5-8-95)		18-20	2	
Room temp #11 (1-27-95)	1.1X	21-23	2.64	
-20, 5 months on P52 (see Rxn#21-23 on P52) got 54% recovery (on P53)		24-26	2	

Exhibit **143**
Appl. No. 09/558,421

°C		SAM	CPM1	ave	ul/µl	% of zero time P122,9
		1	1779.00			
10		2	1970.00	2029	.008	38%
		3	2337.00			
11		4	8375.00		.035	103
		5	8284.00			
		6	8267.00			
1		7	10246.00	9784	.039	105
		8	9851.00			
		9	9556.00			
		10	8959.00			
14		11	9908.00	9484	.037	107
		12	9584.00			
		13	10530.00			
Taq		14	9527.00			
		15	9706.00	10,119	(.04 by definition)	
		16	9859.00			
		17	10773.00			
		18	6924.00			
5		19	7046.00	7065	.028	(was .025 on P53 so >100%
55		20	7219.00			
		21	6156.00			
		22	6520.00	6257	.025	77% (P154,9 is 0 time po (P38,10)
1		23	6095.00			
		24	5038.00			
0°C		25	4980.00		.019	.017/.030 = 63% recovered
55		26	4755.00			see P53
RGD		27	82.00			
		28	119284.00			
		29	121726.00			

To Page N

Witness d & Underst d by m ,	Dat	Invented by	Date
DPolesky	8/11/95	Record d by	7-31-95

Project No._____
130 Book No._____ TITLE Hot vs Cold start PCR w/ Tne (5-

From Page No.__ Is more specific product made and less smear in a hot vs cold star
Look at products at 10, 15, 20, 25, 30, 35 cycles

• start 100ul rxns w/ 2ul enz in order to keep [glycerol] low ⇒ SB
50%, rxns will be 1%, do duplicate hot & cold start rxns

• materials: ~~5x stock of Tricf~~

[A] mix: (5X) { 100mM Tricine pH 9 ⇒ 100ul 1M Tricine pH 9 ✓
5.25mM MgOAc ⇒ 5.25ul 1M MgOAc ✓
425mM KOAc ⇒ 212.5ul 2M KOAc ✓
682.25ul H_2O ✓
1ml

cocktail for 4.5 reactions = 90ul 5X [A] mix ✓
315ul H_2O ✓
[B] 9ul 50pg/ul M13RF ✓
9ul 20μM anchor primer ✓
9ul 20μM 4681 primer ✓
9ul 10mM dNTPs ✓
441ul

98ul B 2ul Tne 0.68u/ul | 98ul B 2ul Tne | 98ul B 2ul Tne | 98ul B 2ul Tne

① ② ③ ④

cold start

hot start - enz a dilutio denatu of 1st
2:40 PM

Tne dilution: 3ul Tne (5-7-95 L:3) 4.84u/ul ✓
27ul Tq SB ✓
30ul of 0.484u/ul Tne

T Pag N

Witness d & Underst d by me, Dat 8/1/95 | Inv nt d by | Dat 7/31/95
R cord d by Pamela Pomb

g N.

nove 10ul after 10, 15, 20, 25, 30, 35 cycles + add 2ul STOP w/ 100mM EDTA
rk to 9600 program 103 = 94°C 1min
35x { 94°C 30sec
55°C 30sec
72°C 2min }
4°C —

% agarose gel
top
—
— → 2/3 1-10c to 1-35c thr 4-35c } 2
2/3 19-10c 22-35c } 2

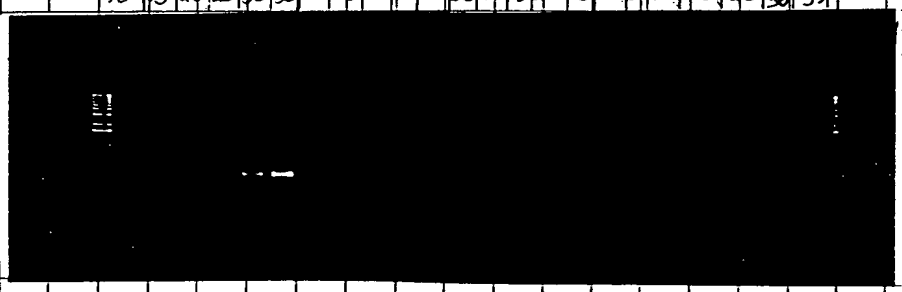
If: Tne (5-7-95 Liz prep) 1.36 u/rxn

Cold Start - duplicates | Hot Start - duplicates

cycle#	10	15	20	25	30	35	10	15	20	25	30	35	10	15	20	25	30	35	10	15	20 25	30	35

buffer = 20mM Tricine
85mM KOAc
1.05mM MgOA

3.1 uL



- 380bp m.3 product

lusion: Hot start did not result in more specific product.
One cold start duplicate rxn failed, no apparent reason.

To Page No.

ed & Understood by me,	Date	Invented by	Date
Bolanos	8/1/95	Recorded by Carolyn Cumby	7/31/95

Project No._____

32 Book No._____ TITLE Addition of genomic DNA to a Tne PCR rxn

rom Page No. 123

same as p. 123 ✱3 except only 1.36u of Tne (5-7-95 will be used per rxn

✱19 90ul D ✓
5.5ul H_2O ✓
2.5ul genomic DNA 5/16/95 HS✱2 ✓
2ul Tne 0.68u/ul p.130

✱20 90ul D ✓
8ul H_2O ✓
2ul Tne

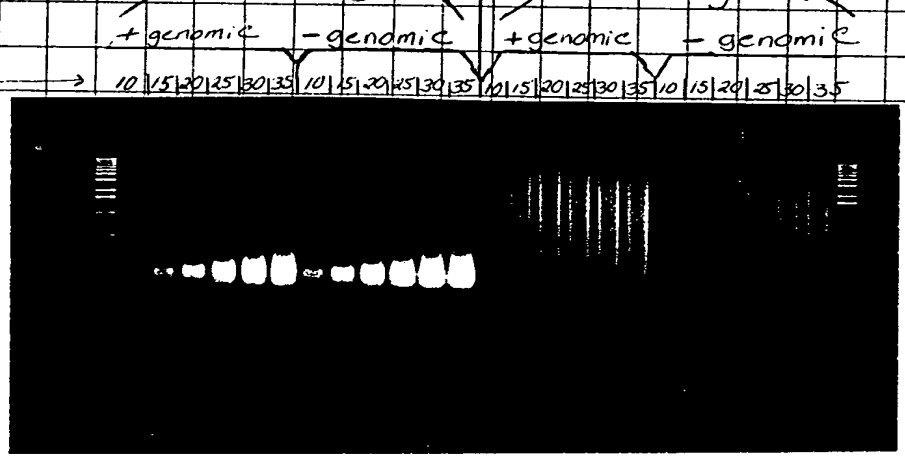
✱21 90ul E
5.5ul H_2
2.5ul g
2ul Tne

✱22 90ul E ✓
8ul H_2O ✓
2ul Tne

10ul aliquots removed after 10, 15, 20, 25, 30, 35 cycles + 2ul stop w/ 100m

Tne 1.36 u/rxn (5-7-95 Liz prep) - no pr

esult:



7/31/95 CC

Conclusion: 1) The smear is visible even after 10 cycles - we shou
run aliquots of earlier cycles to determine when the
smear becomes visible
2) The 1.05 mM Mg^{2+} long smears are more intense with
genomic DNA present than without genomic DNA. T
ay be a real result or it may be variation in rxns - repeat w/ triplicate

of support for
a that genomic
DNA can act as
a bad seed.

To Pag N

n ssed & Understood by me,	Date	Invent d by	Dat
	8/1/95	Record d by Paulson Pomb	7/31/95

Mg^{2+}] titration in a short PCR rxn w/ Tne (5/7/95 Liz)

g N ___

ose: To demonstrate that lowering [Mg^{2+}] shifts the size of DNA products in smear from small to longer ~~[illegible]~~ in a short PCR rxn w/ Tne alone. This result has been observed w/ ~~[illegible]~~ 1.5mM Mg^{2+} vs 1.05mM Mg^{2+}, but the range of concentrations has not been tested. Does the transition occur over a narrow or broad range of Mg^{2+}? What happens w/ 85mM KOAc & 1.5mM Mg^{2+} & we have not tested this condition before

ical expt conditions:

f MgOAc (mM) 0.9, 1.05, 1.1, 1.15, 1.2, 1.3, 1.4, 1.5, 1.7 (9 levels)
f KOAc (mM 50, 85 (2 levels)
emplate and primers for m13 330bp product present
35 cycles, 55°C annealing temp
:.36 u/rxn rxn Tne - 5-7-95 Liz prep
tart rxns w/ Mg^{2+}

erials: 25mM MgOAc → $\frac{25mM}{100mM} \cdot \frac{100ul}{} = \frac{25ul \text{ 1M MgOAc}}{975ul\ H_2O}$

A - 50mM KOAc for 9.5 rxns

19ul 1M Tricine pH 9.0 ✓
23.75ul 2M KOAc ✓
734.35ul H_2O ✓
19ul 10mM dNTPs Cf: 200uM ✓
19ul 20uM 6681 primer ✓
19ul 20uM anchor primer ✓
19ul 50 pg/ul M13RF ✓
1.9ul Tne (5-7-95 Liz prep) ⇒ 1.36 u/rxn
855ul ← 0.8 u/ul

mix B @ 85mM KOAc 9.5 rxns ✓

19ul 1M Tricine pH 9 ✓
40.375ul 2M KOAc ✓
717.725ul H_2O ✓
19ul 10mM dNTP ✓
19ul 20uM 6681 ✓
19ul 20uM anchor ✓
19ul 50 pg/ul m13 ✓
1.9ul Tne 5-7-95
855

	top of gel								no tube 9 - I dropped it		there is a tube 18	12 PM – ~3 PM Lab 16 9600 10.3
	1	2	3	4	5	6	7	8	9	10 – 18		
	90ul									90ul		
	6.4	5.8	5.6	5.4	5.2	4.8	4.4	4	3.2	same series as 1-9		
MgOAc	3.4	4.2	4.4	4.6	4.8	5.2	5.6	6	6.8			

add Mg, mix well & keep on ice til cycling
100ul rxns
stop w/ 1ul STOP w/ 100mM EDTA, run 20ul on 1.2% agarose gel

To Page No.____

d & Und rstood by me, [signature] Date 7/7/95

Invented by [signature]

Record d by [signature] Date 8/1/95

Addition of genomic DNA to a Tne short PCR rxn - 3, 6, 10, 12, 15, 20 cycle aliquot

g N .132

rpose: To determine if adding human genomic DNA to a Tne PCR rxn leads to production of a smear by an earlier cycle # than without genomic DNA. Genomic DNA (200 ng/100 ul rxn) might act like more "bad seed" material and exacerbate the smearing rxn. This experiment was tried on p. 132. Cycle #'s 10-35 were run on the gel. There was an indication that addition of genomic DNA made the smear darker by an earlier cycle #. Now, we are repeating the p. 132 exp in triplicate and looking at even earlier cycle #s.

mmary of experimental cond: 3 rxns w/ human spleen DNA, 3 without
terials: no primers added
start rxns by adding 1.3 ul/100 ul rxn w/ Tne (5-7-95 Lig prep)
@ 55°C annealing temp program 103, Lab 16
200 ng of human spleen DNA/rxn

terials: mix A: for 7.5 reactions = 75 ul 10x PCR buffer ✓
569.25 ul H_2O ✓
15 ul 10 mM dNTPs ✓
15.75 ul 50 mM $MgCl_2$ ✓

Tne (5-7-95 dilution
2 ul Tne
18 ul SB ✓
20 ul 0.1 Tne

top of gel — bottom of gel

	1	2	3	4	5	6
90 ul	—	—	—	—	—	—
5.5 ul	—	—	—	8 ul	—	—
c DNA 2.5 ul	—	—	—	none	—	—
7 ul						
(0.68 ng/ul) 2 ul	—	—	—	—	—	—
-7-95 Lig prep						

ve 10 ul to a tube on ice w/ 2 ul STOP soln in it (100 mM EDTA)
3, 6, 10, 12, 15, 20 cycles, run 10 ul on 1.2% agarose gel
= 6-9: + 3 ul 6-9 c went into 6-9 c stop tube c = cycle #

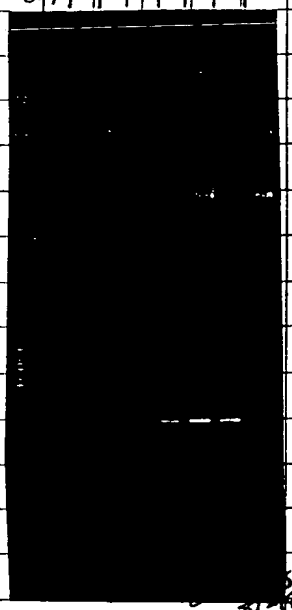
See result on p. 134

To Page No. 134

ed & Understood by me,	Date	Invented by	Date
Sarah a Polan	5/7/95		
		Recorded by Carolyn Coons	8/1/95

TNE (5-7-95 Liz prep) 1.36 u/rxn, 100ul rxn w/ template + primers

MgOAc (mM) 0.9 | 1.0 | 1.1 | 1.15 | 1.2 | 1.3 | 1.4 | 1.5



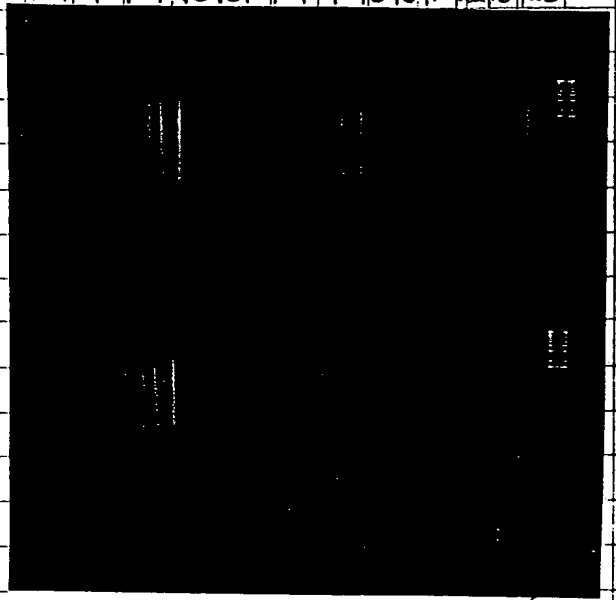
50mM KOAc

85mM KOAC.

- 1.3mM MgOAc was optimal for making product - note that we have made prc w/ 1.05mM Mg^{2+} in earlier e when glycerol → ~~buffer~~ 5% also pro p.86
- 50mM KOAc is not sufficient for produ formation, but 85mM KOAc is - valu between 50 + 85 have not been teste
- The size of DNA products in sme does vary from small to longer as $[Mg^{2+}]$ varies from 1.5 - 0.9mM Mg

triplicate rxns w/ TNE (5-7-95 Liz prep) (see p135 for reaction)

	replicate 1					replicate 2					replicate 3				
cycle #	3	6	10	12	15	20	3	6	10	12	15	20	3	6	10	12	15	20



+ 200ng human spleen genomic DNA per 100ul rxn

no genomic DNA

Conclusion:
There maybe a l amount of contam DNA still in RNA in TNE prep - 3 cycles needed see smear.

e. coli 5/2/95

T Pag

Witn ss d & Underst od by m , Dat 8/7/95 Inv nt d by Dat 4-2-97

R c rded by Pomb

Project No._____

136 Book No._____ TITLE Mg^{2+} titration in long PCR w/ Taq: Tne

From Page No.__

purpose: To determine if Mg^{2+} controls long PCR smear size and to see if the smear is primer/template independent. Note that Long PCR kit uses different buffering components than we've been using for si

In a single Tne enz. PCR of 350 bp product, 1.2mM Mg^{2+} - 1.3mM is at the center of transition from small to long smear is optimal for product formation if 85mM KOAc present.

do: 7 levels of Mg^{2+}, ± target and primers, 2 ratios of Taq: Tne

materials: LTI:S Taq Long PCR system + Kalas recommendations for 7-22-95 Tne prep

dilution of Tne in Taq SB: 2ul Tne (7-22-95 prep, 19 u/ul)

① 59.64ul Taq SB ✓ 1:30.8 2

mix

② 2ul of dilution ①

59.64ul Taq SB 1:30.8 2

61.64ul of 0.024/ul Tne

enzyme mixes:

final Taq (u):	Tne (mu)	Taq (5u/ul)	Tne (0.024/u)	SB
→ 1	1	4 10	4 2.5	5 3
1	10	4 10	40 25	8 1.
1	2	4 5	4 2.5	7 1

mix 1 with primer and template for 16 rxns: 16ul 10mM dNTP

16ul primer mix 1

KSU 2 DNA / 2mg/ml, 8ul Bio H_2O 16ul genomic temp

272ul H_2O

320ul

mix 1 without primer + template, 16 rxns:

16ul 10mM dNTP

304ul H_2O

320ul

T Pag N

Witn ss d & Und rst d by m, Dat 8/7/95 Inv nt d by Dat

R c rd d by 8/2/95

g N. — Mg2+ mM

2 (1:1 TT) 1 for 5 rxns = 50 ~~20~~ ul 5x A ✓ ,2.5ul
Taq:Tne 0 ~~30~~ ul 5x B ✓
5 5 ul 1u Taq : 1mU Tne (7-22-95 R.L. prep)
95 ~~95~~ ul H_2O
~~150~~

2 (1:1 TT) 1 mM Mg^{2+} = 45 ul 5x A ✓
5 ul 5x B ✓
5 ul 1u Taq : 1mU Tne ⇒ (~~a 100ul H_2O enz mix~~ → 2.5 add)
add H_2O 1st 95 ul H_2O

2 (1:1 TT) 1.2 mM Mg^{2+} = 40 ul 5X A ✓ ~~→ Barry's aliquots~~
10 ul 5X B ✓
5 ul 1u Taq : 1mU Tne
95 ul H_2O

2 (1:1 TT) 1.3 mM Mg^{2+} = 35 ul 5X A ✓
15 ul 5x B ✓
5 ul 1u Taq : 1mU Tne
95 ul H_2O

2 (1:1 TT) 1.4 mM Mg^{2+} 30 ul 5x A ✓
20 ul 5x B ✓
5 ul 1u Taq : 1mU Tne
95 ul H_2O

2 (1:1 TT 1.5 mM Mg^{2+} 25 ul 5x A
25 ul 5x B
"

2 (1:1 TT) 1.6 mM Mg^{2+} 20 ul 5x A
30 ul 5x B
1 mix 2 "

ke each of these mixes again with the 1:10 TT mix
d a mix 2 (1:2 TT) 1.6 mM Mg^{2+}

To Pag No.

ed & Understood by me, [signature] Date 8/7/95
Invented by [signature] Date 8/2/95
Recorded by [signature]

Project No._______

Book No._______ TITLE __________

From Page No.__

*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
mix 1 w/ template + primers	20						|								20						|							20
mix 1 no primers on template								20						|								20						|	
mix 2 (1:1) 1	30							30																					
1.1		30							30																				
1.2			30							30																			
1.3				30							30																		
1.4					30							30																	
1.5						30							30																
1.6							30							30															
mix 2 (1:10) 1															30							30							
1.1																30							30						
1.2																	30							30					
1.3																		30							30				
1.4																			30							30			
1.5																				30							30		
1.6																					30							30	
mix 2 (1:2) 1.6																													30

10:30 am Lab 15 94°C 30 sec

35 cycles { 94°C 30 sec; 60°C 30 sec; 68°C 10 min } ~6.5 hr

4°C

stop w/ 7 µl stop soln containing 100mM EDTA (p.79)

20 µl run on 0.7% agarose gel, 190V 6:30 pm - 7 pm

Witnessed & Understood by me, [illegible] Date 8/7/95

Invented by [illegible]

Recorded by [illegible] Date 8/2/95

To Page No.

Project No._____ 32 P end label Pri 2 --- Pri 2

140 Book No._____ TITLE for test of decreasing primer len

From Page No.__

mix for 14 labeling rxns: 23 ul $^{3-2}$P ATP (3.33 uM) ref 8/4/95 (6.66 pm per
25 ul 5x kinase buffer
4.2 ul PNK - new lot
50.56 ul H_2O
146 ul

rxns = 1.66 ul of each oligo - Fidel pri 2, 3, 4, 6, 8, 10, 12, 14, 16, 1
← 10 uM stocks
(16.6 pmol primer tot
8.34 ul mix
10 ul in 9600 tubes
37°, 30' in Lab 14 9600
55°C, 5' "
cool to 4°C before opening tubes

add 2 ul 10 uM Fidel temp dT = 20 pmol total
80°C, 5'
cool to Room temp, 15 min in 9600

add 64.7 ul 10 mM Tris pH 8.0
stored at -20°C overnight

use 42/100 reaction for 10 mM primer

$\frac{\text{template}}{\text{pri}} = \frac{20 \text{ pmol}}{16.6 \text{ pmole}} = 1.2$

To Pag N

Witn ss d & Und rsto d by m , Dat 8/7/95 Inv nt d by Dat 8-7-95

R cord d by

Exhibit 147
Appl. No. 09/558,421

Project No. ______
Book No. ______

142

TITLE Test extension of short primers Δ [Taq], Δ length of primer

From Page No. ___

not done

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

^{32}P fid pri 6 - fide / Temp — 2 ⟶
" 8 — 2 ⟶
" 10 — 2
" 12
" 16
" 20
(P140, 250nM primer)

Mix A — 46 μl ————

Tag μ/l ~~sil buffer~~ 5B

.0016 — 2 2 2
.008 — 2 2 2
.04 — 2 2 2
.2 — 2 2 2
1 — 2 2 2
5 — 2 2

50 μl

70°C ~~20 min~~ 22 min
stops with 25 μl agile seq stop solution
load 3 μl on 25% PAGE
(same as P155, 7)
2000 V (gets ~11 mA) for 3 hr

.0032 units (e.g. tube #1) 21 pmol nt in 20 min
(based on units a max efficiency)

input primer is 1 pmol 42 mer
= ~20 pmol nt incorp for 20 nt ssDNA seq

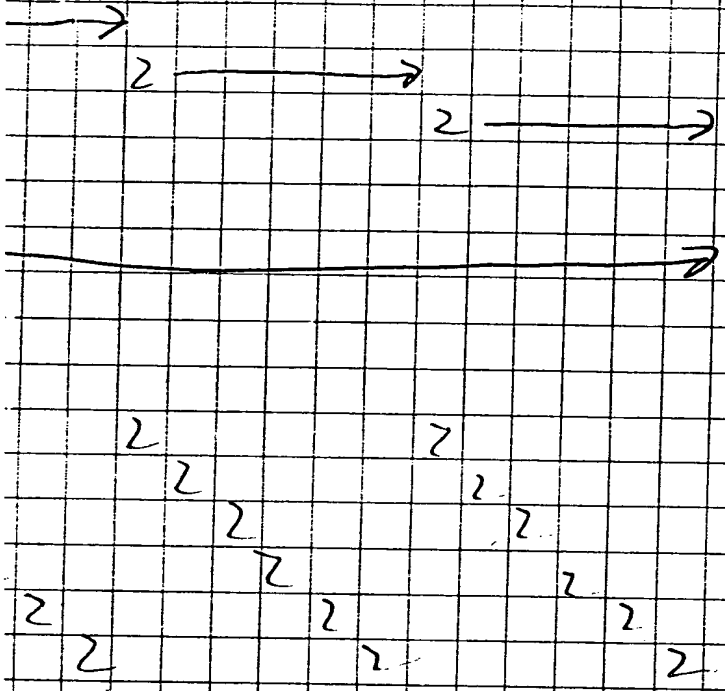
To Page No.

Witnessed & Understood by me, [signature] Date 8/7/95
Invented by [signature] Date 8-3-95
Recorded by [signature]

g No.__

22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

10 nM primer Cf
(1 pmol primer total/50 µl Rx



(for 40 Rxns)

Mix A

H2O 1.5-40 ml ✓
10X PCR buffer 200 ✓
50 mM $MgCl_2$ 60 ✓
10 mM dNTPs 40 ✓
1.5-340 ml

use 46 µl/Rxn

Cf = 1.5 mM $MgCl_2$

200 µM dNTP Cf in Rxn.

To Page No.____

& Understood by me,

Date 8/7/95

Invent d by

Recorded by Carolyn Combs

Date 8-7-95

PAGE 144 OF NOTEBOOK WAS BLANK

g N.

Results:

1. primer length 10 and longer are extended by Tag.

2. Will Test Tine vs Tag next to see if Tine does better than Tag

To Page No.

ed & Understood by me,

Date 8/14/95

Invented by

Recorded by

Date 8-3-95

rom Page No.__

32P fid pri 6 · fidel Temp
" 8
" 10
" 12
" 14
" 16
(P140 250 nM primer) *preheated to 70°C w/mix A*

Tne diluted in SB 7/22/95 R.L.
2.0 1.56 u/l
0.0625 u/l
0.25 u/l
1 u/l

Taq 5/31/95 Lin
0.0156 u/l
0.0625 u/l
0.25 u/l
1 u/l

Mix A (P.143) 46 µl
Vf = 50 µl

20 min 70°C stop with 25 µl cycle seq stop solution

heat to 90°C, 5 min before loading

25% PAGE run as P142 and 155 ; ran 3 hr 2000 V

mix A p.143 scaled up 1.5X = 2.31 mL H_2O
300 ul 10x PCR buffer (from Kala)
90 ul 50mM $MgCl_2$ (made from 1M 2/15 STD → 50 ul 1M in 950 ul H_2O)
60 ul 10mM dNTPs
2760 ul

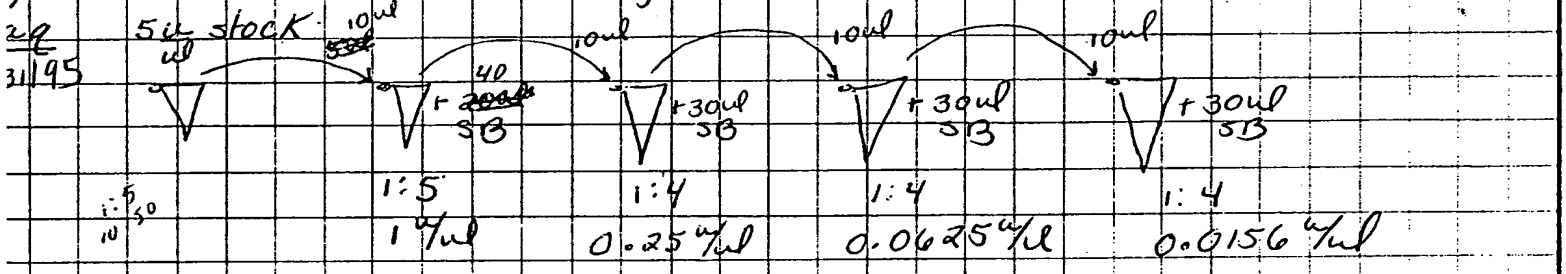
To Page No

Witness d & Understood by m, Dat 8/7/95
Invented by Dat 8-4-95
Recorded by Dawson Poole

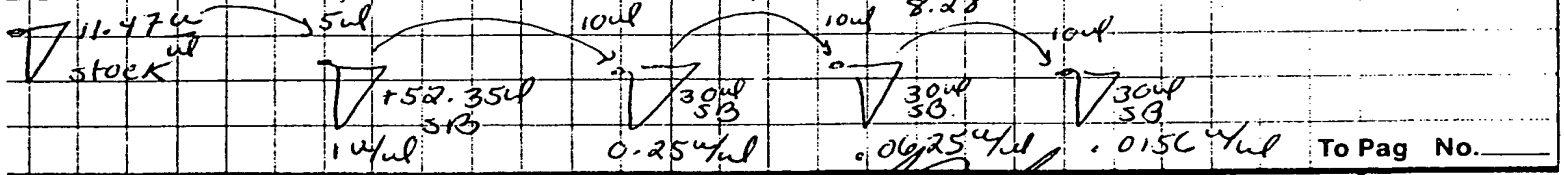
ge No.

yme dilutions: in Taq storage buffer — 12/7/94

Taq 3/1/95



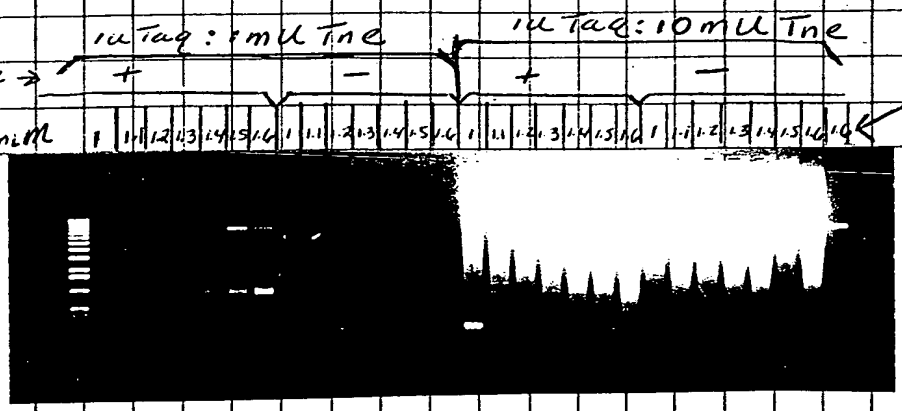
ze 1st normalize to Taq units p. 127 $\frac{19}{8.28} \times 5 = 11.47$ u/ul (Taq was thought to be 5 u/ul



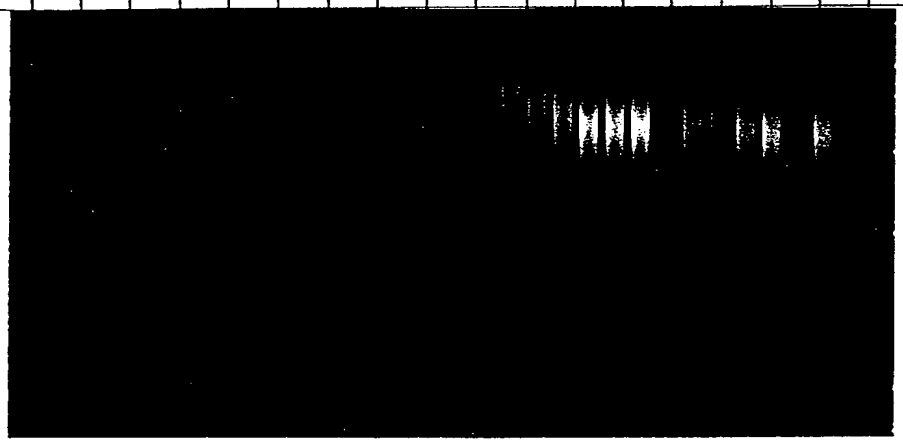
To Pag No.____

ed & Understood by me,	Date	Invented by	Date
Laura Palmer	8/7/97		8-4-97
		Recorded by Carolyn Combs	

g N .__



8/15/95 cc



8/15/95 cc

There are 2 kinds of nonspecific products

1) primer/target dependent nonspecific products, which are seen with both 1munit and 10munits Tne. The products are discrete bands, mostly ≤ 1Kb. The ~~taten~~ yield of these nonspecific products, as well as the specific 7.5Kb product increase as $[Mg^{2+}]$ increases. The highest ratio of specific to nonspecific product occurs at 1.5 mM M

2) primer/target independent products. The products form an intense smear from wells down to ≤ 200bp. As $[Mg^{2+}]$ increases, the size of products in smear decreases and the smear becomes more intense. This is the same Mg^{2+} affect that was observed in a short PCR with Tne alone.

To Page No.____

ed & Understood by me,	Date	Invented by	Date
Rebecca Polanco	8/7/95	Recorded by Carolyn Combs	8/15/95

[redacted]

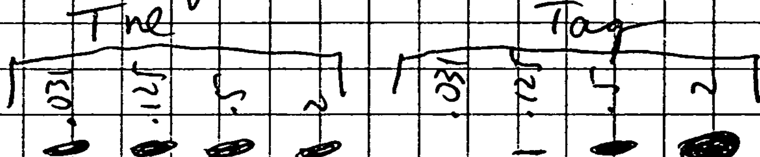
Project No.______
Book No.______ TITLE ______

From Page No.__ Results Data on P 151, 152

for primer length 12, 14, 16 the first appearance of run off happens at 4 - 16 X less Tne units than Taq units (~~based~~ units based on Taq unit assay P. 127

at the highest [pol], Tne and Taq have ~ equal extension for 12, 14 nt primers and Taq has greater yield for 16 nt primer at highest level

ie. for 16 mer primer, Tne again shows first appearance of ~~runoff~~ run off but Taq has greater yield at 1/2 units/enzyme ∴



∴ less Tne is needed to extend a primer. Maybe Tne is more processive
That is consistent with Taq giving good extension (equal or better than Tne) at high and ~~[illegible]~~ could explain how Equal units of Taq, Tne for DNAse I treated gapped DNA act differently for primer extension

To Page N

Witnessed & Understood by me, Date | Invented by | Date
[signature] 8/7/95 | [signature] | 8-7-95
Recorded by

g N — see p. 140

d-label Fid 16: ~~4 ul~~ $^{33}P\gamma ATP$ ref 8/4/95
~~4~~

8.08 ul H_2O ✓
4 ul 5x Kinase buffer ✓
3.32 ul 10uM Fid 16 oligo ✓ (~~33.2~~ 16.6 pmol)
4 ul $^{32}P\gamma ATP$ ref 8/4/95 (3.33uM, 4.66 pmol/um)
0.6 ul PNK

20ul in 96oo tube
37°C, 30min ✓
55°C, 5min ✓
cool to ~4°C ✓

+ 4ul Fidel Temp, 10uM 42mer (40pmol)
50°C, 5min
cool for 15min to RT in PCR machine
+ 189.4 ul 10mM Tris pH8.0

store at -20°C

To Page No.

ed & Understood by me,	Date	Invent d by	Date
Polanp	8/14/95	Recorded by Carolyn Comb	8/7/95

PAGES 150-151 OF NOTEBOOK WERE BLANK

32P 42 mer for 3'exo assay of 3'exo(-) Klenow fragment

g N.—

Fidel Template 10μM (42 mer) 5.8 μl ✓ 5 Pmol total

32P ATP 7 ✓

5x Kwiaw buffer 7 ✓

PNK 10u/l 2 ✓

H2O 13.2 μl ✓
35

37°C, 30'

70°C, 5'

1M KPO4 pH 7.0

1M Kmonobasic 1.3 ml

1M K dibasic 8.7 ml

Vf = 10 ml

To Page No.____

d & Understood by me,	Date	Inv nt d by	Date
[illegible] Roland	8/14/95	R corded by	8-8-95

Project No.
52 Book No. TITLE Primer extension: time course with 4 [enz], Taq vs Tne Fid16 primer

rom Pag No.

purpose: In the last primer extension experiment, ~4x less Tne th Taq was able to extend the Fid16 primer to a full leng 42mer product. p.150. The highest level of Taq (2u), that was tested, made even more full-length product than the highest level Tne tested (2u). ~~Today's expt.~~ The purpose of today's e is to confirm the results of p. 150 and to determine whether Tne = Taq have different affinities for DNA/primer bi or if they are the same but Tne is better at extendin after a stop. → the time course - no enz rxn will reveal if Tne cures a stop sequencing rxns and the Fid16 primer without enzyme will also be run on the 25% gel.

materials:

PCR/Mg^{2+} mix to mix with enzyme prior to beginning rxns:

		conc
25 ul	10x PCR mix ✓	1.25x
7.5 ul	50mm $MgCl_2$ ✓	1.25x
167.5 ul	H_2O ✓	
200 ul		

(2) ✓

↳ use 16 ul ✓
+ 4 ul of enz. dilution in SB ✓
20 ul - 1x PCR buffer = 20mm Tris 8.4, 50m
tubes 1-8 1x $MgCl_2$ Cf = 1.5mM

(1) mix A for 10 rxns: 80 ul 10x PCR buffer ✓
636 ul H_2O ✓
24 ul 50mm $MgCl_2$ ✓
20 ul 10mm dNTPs ✓
40 ul ^{32}P Fid16 annealed to Fidel Temp p. 14
800 ul
Keep at 70°C

✓ enzyme dilutions in Taq storage buffer: same as on p. 147
same preps of Taq & Tne

(3)

✓ 5 ul STOP soln from cycle sequencing kit in 9600 tubes 1-40

To Page N

Witnessed & Understood by me,	Dat	Invented by	Date
DPolanp	8/14/95	Record d by Paula A. Pavlik	8/8/95

g N .__

#	1	2	3	4	5	6	7	8
ubes	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-46
z/95		all prewarmed to 70°C						
Tne mix	20							
PCR er+Mg^{2+}								
Taq mix		20						
l Tne mix			20					
l Taq mix				20				
¼l Tne mix					20			
¼l Taq mix						20		
¼l Tne mix							20	
¼l Taq mix								20

rxns w/ 80ul of mix A prewarmed to 70°C, triterate w/ p200

ve 10ul of rxn to a 9600 tube containing 5ul STOP at 1min, 2min, 5min, 10min, 20min - on ice

t to 80°C, 5min before loading ~~3~~ 1.5 ul on 25% gel

er of loading: on 25% urea gel

ft ATGC no enz Fid16 ~~40~~ 31-40 → 21-30 → 11-20 → 1-10 not loaded δATP

↓ 16ul 1.25x PCR buffer
4ul storage buffer
+ 80ul mix A

↳ removed 10ul + 5ul stop - from cycle sequencing kit

no enz Fid16 | no enz Fid16 | no enz Fid16

Diluted 10x to 1:10 3ul/ul

↳ .3ul + 2ul H_2O + 1ul STOP load 1.5ul

ed on 8% gel in same order loaded 1.5ul

To Page No.____

d & Understood by me,	Date	Invented by	Date
olamp	8/15/95	Recorded by Carolyn Comb	8/8/95

Exhibit **152**
Appl. No. 09/558,421

52 Project No.______ Book No.______ TITLE ______

From Page No.__

Taq PCR conditions

	10 Rxns	A	B
BSA 20mg/ml	✓	2.5 µl	BSA=0.T
1M KH_2PO_4 pH7.6	✓	25	5
10X PCR buffer	✓		50
50 mM $MgCl_2$	✓	70	70
50% sterile glycerol	✓	140	1
H_2O (sterile)	✓	232.5	350
^{32}P 42mer (fidel Template P61	✓	10	10
		480	480

*see P 74-75
*Beckman-Dickerson conditions ... Taq PCR conditions

3' P48-mer no enz

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
(A)	48µl →																	
(B)										48µl →								
Klenow exo- lot CK041 75 u/λ																		
dil 25 u/λ	2									2								
5 u/λ		2									2							
1 u/λ			2									2						
Klenow exo(-) lot EJP41 130u/λ																		
dil 25 u/λ				2									2					
5					2									2				
1						2									2			
Klenow (+) exo 0.6 u/λ							2									2		
0.06								2									2	
0.006									2									2

dilutions with Taq dil buffer

start 1:2

3 hr,

25 µl cycle stop sol

load 3 µl 8% PA

To Page No.

Witnessed & Understood by me, [signature] Date 8/14/95

Invented by [signature] Date 8-9-95

Recorded by

P63, 10

r m: Hartman, Chris
o: Lasken, Roger
c: Rashtchian, Ayoub
ubject: Exo minus Klenow Boston
at : Monday, August 07, 1995 3:40PM
ri rity: High

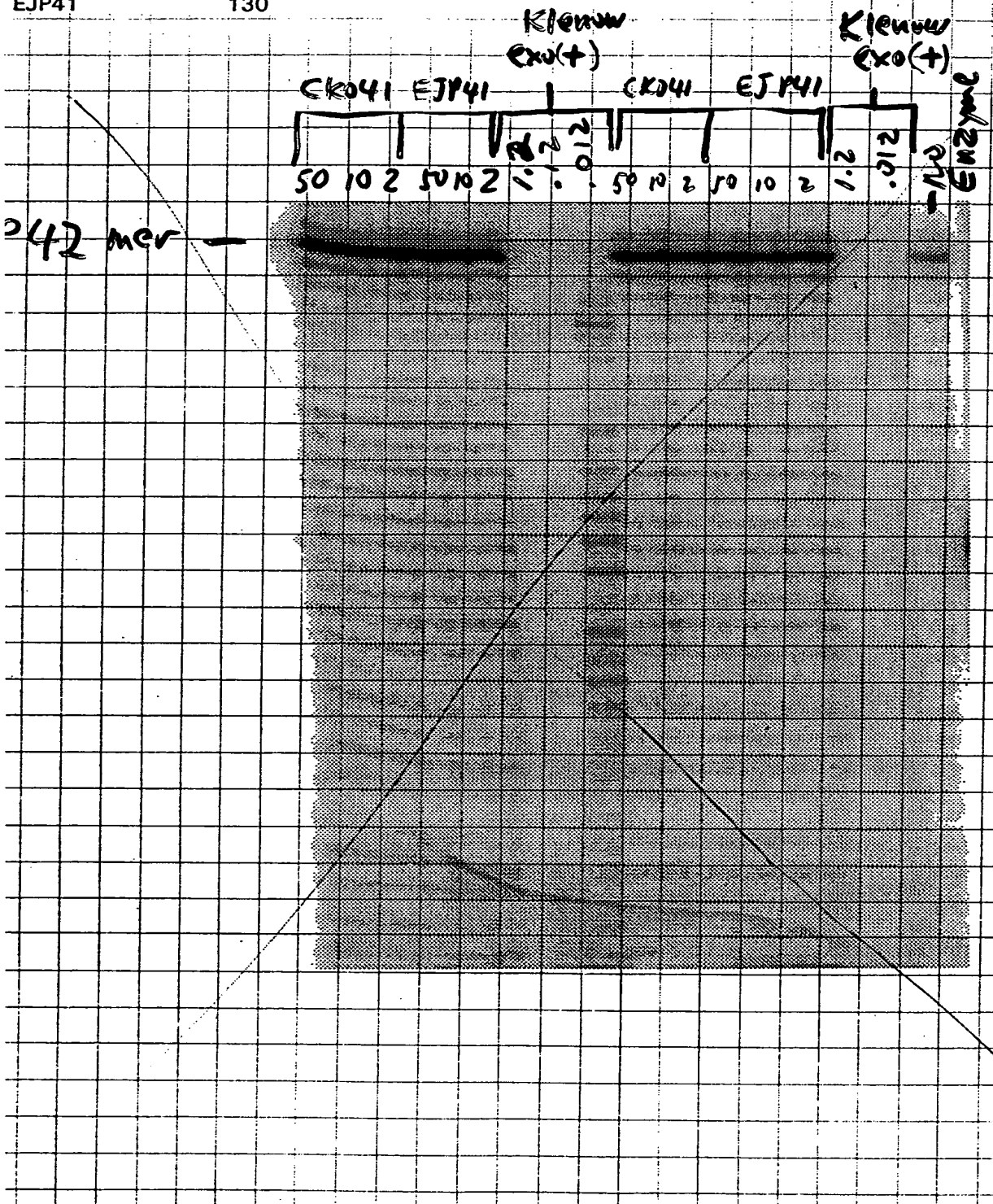
roject No._____ Book N ._____ 63

Dickinson Conditions

Taq pol buffer

oger, The unit values for the bulk exo minus klenow are as follows:

Lot No.	U/ul
CK041	75
EJP41	130



To Page No.____

d & Und rstood by me,	Date	Invented by	Date
Polanp	8/14/95	Recorded by	8-14-95

Project No.______
Book No.______
TITLE cont'd 33 correct · mp19 (follow p 17, 9 for 500 pmol

From Pag No.__

33 correct (P138, 9) 20 μM ✓ ~~2.18~~ 4.36 μl ~~43.6~~ 87 p[mol] pr[imer]

M13 mp19 sDNA(+) ✓ 200 μl 21.8 pmo[l] total

1 m Tris pH 7.5 ✓ 10.6 μl (100 pri[mer]/temp[late])

Vf 212.78 μl 50 mM Tris

∴ 0.1025 pmol circle/μl ⇒ 743 pmol nt/μl
use 2 μl/50 μl Rxn for 1.5 nmol nt/Rx[n]

Mix A

(33.4 Rxns)

Taps MgCl₂ KCl (of P' 120, 9)	~~103~~ μl (use 15 μl next time)	✓	
^{32}P dCTP 10 mCi/ml 3000 Ci/mmol	3	✓	
dATP 10 mM	33.4	✓	(200 μM)
dCTP 10 mM	33.4	✓	
dGTP 10 mM	33.4	✓	↓
rTaq 5 u/μl	66.8 μl	✓	10 u/50
33·mp19 743 pmol nt/μl	66.8	✓	1.5 nmol nt
H_2O	1.163 ml	✓	
	1.503 ml		

use 45 μl/50 μl Rxn

T Pag

Witnessed & Understood by me, [signature] Dat 8/14/95
Invented by [signature] Dat 8-[illegible]
Recorded by

ge N			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
A	4 μl →																												
CC703 β		—																											
M			5																										
				5																									
					5																								
						5																							
							5																						
								5																					
3707	H_2O																												
L	—							—																					
M	40	→ 40								5																			
	40	→ 40									5																		
	40	→ 40										5																	
	40	→ 40											5																
	25	→ 50												5															
82 8.3 μl + 5 μl 10 mM 4 dNTP															5														
(+Taq) P121.9																—													
	40	→ 40															5												
	40	→ 40																5											
	40	→ 40																	5										
	40	→ 40																		5									
	25	→ 50																			5								
~~2070~~ μl + 30 μl ~~200~~ μM in mix B																						5							
A (-)Taq																							—						
																								5					
																									5				
																										5			
																											5		
																													5
O			5							5							5							5					

(stability study began at 4°C on 1-20-95 ∴ samples are 6 months and 20 days old)

220 (@ 1.1X)

spot 20 μl on GFC

Vf = 50 μl assemble on ice → 1 min 70°C → ice bath → 10 μl 0.5 M EDTA

To Page No.______

Witnessed & Understood by me, [signature] Date 8/14/95

Invented by [signature] Date 8-1-95

Recorded by

Project No. total dTTP
Synthesis Book No. pmol TITLE Results P6T
(pmol)

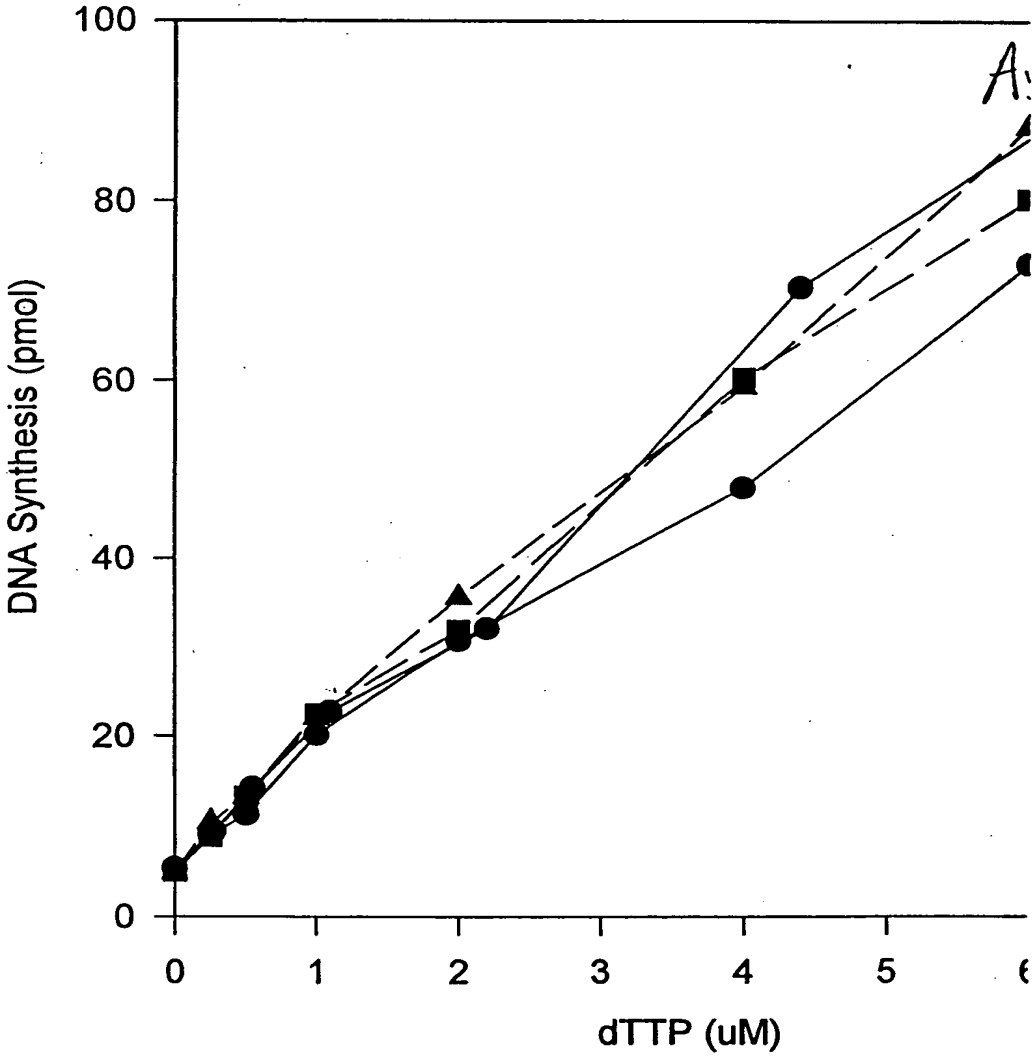
0	82.60	5.4	0	
.25	137.80	9.1	12.5	18%
.5	172.00	11.4	25	11%
1	303.80	20.1	50	10
2	463.40	30.7	100	7.5
4	725.00	48	200	6
6	1103.80	73	300	6
0	76.00	5.0		
.25	134.40	8.9		
.5	201.00	13.3		
1	339.20	22.4		
2	482.00	31.9		
4	910.20	60.3		
6	1211.80	80.2		
0	82.20	5.4		
.25	145.80	8.6		
.5	216.20	14.3		
1	344.60	22.8		
2	485.00	32.1		
4	1062.00	70.3		
6	1408.00	93.2		
0	74.00	4.9		
.25	158.40	10.5		
.5	201.00	13.3		
1	332.60	22		
2	538.20	35.6		
4	896.00	59.3		
6	1333.20	88.3		
0	57.20			
	72558.92			

assuming 1/4 incorp is T
(ie 9.1 pmol / 4)

1.5 nmol at max

∴ 5% on 352 at max

200 uM dATP, dGTP, dCTP



To Pag

Wil rstood by me,

Dat 8/14/95

Invented by

R corded by

Dat 8-9-95

Processivity of Taq, Tne, and Ultma

g N. __

xtension of 33-mer correct primer annealed to m13mp19 ssDNA
serial enz dilutions, 2 min extension and 1 10min endpt extension
2 units — 0.0078 units in 50ul/rxns, reactions started w/ 2ul enz.
?/95

action cocktail for 35 rxns = 175 ul 10x PCR buffer
1347.5 ul H_2O
52.5 ul 50mM $MgCl_2$
35 ul 10mM dNTPs
70ul ^{32}P-33mer correct annealed to m13mp19 - the Kinase rxn was done as on p. 12 NB 10, then 66 ul of m13 ssDNA added 0.26 ug/ul m13 stock
1680ul

te: 42 ^{32}P primer : 1 m13 circle
* mistake, see 8/11/95
were 2ul of the labeled & annealed primer was diluted w/ 70ul m13
" ^{32}P 33 correct : mp19 "
This was 42 pri / circle - see P/64 where more mp19 added to get 1 pri/circle

zyme dilutions in Taq SB
Taq, 5/31/95, 5 u/ul — 2 fold dilutions
5u/ul → 4ul → 16 ul SB (1:5, 1 u/ul) → 10ul → +10ul SB (1:2, 0.5 u/ul) → 10ul → 10ul SB (0.25 u/ul) → 0.125 → 0.0625 → 0.0313 → 0.0156 → 0.0078, 0.0039 u/ul

unit assay on 7/31
ne 7/31/95, 11.47 u/ul - this value is normalized to Taq p. 147 (RL's Tne of 7-22-95)
11.47 u/ul → 5ul → +52.35 ul SB → then, serial dilutions made in the same way as for Taq
1:11.47
= 1 u/ul

Ultma 6 u/ul — Lot 0643 12/31/95. Perkin Elmer
6u/ul → 4ul → 20ul SB (1u/ul) → same dilutions as for Taq & Tne, but Ultma units are not normalized to Taq units.

To Page No.______

Read & Understood by me, [signature] Date 8/14/95
Invented by
Recorded by Carolyn [signature] Date 8/9/95

Project No._____

156 Book No._____ TITLE _____

From Page No.__

- ~~a~~ 48ul mix in a 9600 PCR tube, preheated to 70°C
- reactions were started by adding 2ul of enz w/ P2 and triterating w/ P200
- after 2 min at 70°C, in 9600, rxns were stopped w/ 25ul Cycle sequencing Stop solution and kept at -20°C overnight prior to loading on 8% gel

Tubes	1 — 10	11 — 20	21 — 30
	Taq	Tne	T.m.a
	0.0078 → 20units	0.0078 → 20units	0.0078 - 20uni

The 20units rxns were incubated for ~~20~~ 10 min; while all the other rxns were incubated for 2 min

T Pag N

Witness d & Understood by m,	Dat	Invented by	Dat
	8/14/95	Recorded by Dawson Powell	8/9/95

Project No._____

158 Book No._____ TITLE Extension of 16-mer by Taq + Tne with Δ MgCl + Δ KCl

From Page No.__

general overview of conditions tested:

Fix $MgCl_2$	Δ KCl (mM)			
1.05 mM	0	25	50	85
1.5 mM	0	25	50	85

Fix KCl 50mM	Δ MgCl (mM)
	1, 1.2, 1.5

each condition tested w/ 0.0312u Taq + Tne, 2.0u Taq + Tne in 50ul rxns at = for 20min

= 44 rxns

for 1, 50ul rxn:
- 1ul 1M Tris ~~8.4~~ 8.5 Cf = 20mM
- 41.08ul H_2O
- 1.42ul 3M KCl for 85mM Cf
- 1.5ul 50mM $MgCl_2$ for 1.5mM Cf
- 1ul 10mM dNTP Cf = 200uM
- *2ul ^{32}P primer on Fid Temp Cf = 10nM
- 2ul enz to start rxn

50ul

* note that real PCR buffer is pH 8.4

*End-labeled primer as on p. 149

mix A for 50 rxns:
- 50ul 1M Tris, pH 8.5 ✓ ✓ 22.5 ✓
- 1875ul H_2O ✓ ✓ 843.75
- 50ul 10mM dNTP ✓ ✓ 210ul FHC 705 FE 370 V 22.5 ✓
- 100ul ^{32}P primer annealed to Fid Temp

2075ul

T Pag N

Witn ss d & Understood by m ,	Date	Inv nt d by	Dat
DDolan	8/14/95	Record d by Dandan Pomb	8/10/95

No.___

the expt w/ varied KCl

B w/ 1.05 mM $MgCl_2$
rxns
705.5 ul A
17.85 ul 50mM $MgCl_2$ ✓✓
7.65 ul H_2O ✓✓
731 ul

C w/ 1.5mM $MgCl_2$
705.5 ul A
25.5 ul 50mM $MgCl_2$ ✓
731 ul

same but use 180.6 ul C for each

2 rxn →
- 180.6 ul B + 21 ul H_2O ✓ C_f = 0mM KCl → 48 ul/rxn ✓
- 180.6 ul B + 21 ul 250.6mM KCl C_f = 25mM KCl /rxn ✓
- 180.6 ul B + 21 ul 501.2mM KCl C_f = 50mM KCl
- 180.6 ul B + 21 ul 852mM KCl C_f = 85mM

erial dilution of 3M KCl stock: ✓

3M —50ul→ +126ul H_2O —100ul→ +70ul H_2O —100ul→ +100ul H_2O

3M | 852mM | 501.2mM | 250.6mM

To Page No.____

& Understood by me,
Date 8/14/95
Invented by
Recorded by
Dat 8/16/95

From Page No.__

for expt w/ varied $[Mg^{2+}]$'s

for 14 rxns [D] = 581 ul A
11.62 ul 3m KCl ✓ Cf = 50mM KCl/rxn
9.38 ul H_2O ✓
602 ul

for 4.2 rxns

180.6 ul D
+ 21 ul 10mM Mg^{2+}
↑ use 48 ul/rxn
Cf = 1mM Mg^{2+}/rxn

180.6 ul D
+ 21 ul 12mM Mg ✓
Cf = 1.2mM Mg/rxn

180.6 ul D
+ 21 ul 15mM Mg^{2+}
Cf = 1.5mM Mg

dil of 50mM MgCl stock: ✓

50mM → 50 ul + 116.5 ul H_2O → 15mM

100 ul + 25 ul H_2O → 12mM

100 ul + 20 ul H_2O → 10mM

Cozyme dilutions in Taq storage buffer (SB):

Taq, 5/31/95 stock 5 u/ul (hot real units)

5 u/ul → 10 ul + 40 ul SB → 1 u/ul → 10 ul + 631 ul SB → 0.0156 u/ul

Tne, 7/22/95 stock = 11.47 u/ul, normalized to Taq p. 127 + 147

11.47 u/ul → 5 ul + 52.35 ul SB → 1 u/ul → 10 ul + 631 ul SB → 0.0156 u/ul

To Pag N

Witnessed & Understood by me,	Dat	Invented by	Dat
DDPolanco	8/14/95	Recorded by [illegible]	8/10/95

g N.__

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Mg^{2+} oKCl	48				48	48			48				48											
25KCl		48				48				48				48										
50KCl			48				48				48				48									
85KCl				48				48				48				48								
oKCl																	48				48			
25KCl																		48				48		
50KCl																			48				48	
55KCl																				48				48
1 Mg																								
1.2Mg																								
1.5Mg																								

follow blue ink

w/ul Tne 7/22/95	A	2	2	2	2	2															2	2	2	2	
w/ul Tag 5/3/95		2				2				2				2										
Tne																								
56 w/ul Tag					2	2	2	2													2	2	2	2
Tag			2				2				2			2										
Tne									2	2	2	2												
Tag				2				2				2	2	2	2	2								

To Page No.____

d & Understood by me,

Polarp

Date 8/14/95

Invented by

Recorded by

Date

Project No._____

162 Book No._____ TITLE _____

From Pag No.__

	25	26	27	25	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
			✓				✓			✓			✓			✓			✓
.5mg oxcl	48				48														
25xcl		48				48													
50xcl			48			48	48												
55xcl				48		48		48											
50xcl .1mg									48			48			48			48	
1.2mg										48			48			48			48
1.5mg											48			48			48		
.0156 Tne									2	2	2								
.0156 Taq												2	2	2					
1 Tne	2	2	2	2	—										2	2	2		
1 Taq					2	2	2	2										2	2

1700V, 40mA 12 pm — 12:50 pm

T Pag N

Witn ss d & Underst d by m , D Polanco

Dat 8/14/95

Inv nt d by

R cord d by Pauline Pond

Dat 8/10/95

Exhibit 155
Appl. No. 09/558,421

164

Project No.________

Book No.________

TITLE Redid processivity of p155 expert expand lower [pol] range and correct error in pri/pol from 42 to 1 here) :o

From Page No.___

^{32}P 33 correct • mp19 p155 2 μl

mp19 0.26 μg/λ 70

70

Vf 70 μl

7.6 pmol circ total now have pri/circle = 1

mix A

	70 μl
10 x PCR buffer	175 μl
H_2O	1347.5 μl
50 mM $MgCl_2$	52.5 μl
10 mM dNTP	35
	Vf = 1680 μl

^{32}P 33. mp19 ~ 0.22 pmol circ per 50 μl Rxn

(preheat to 70°C
→ 47 μl mix A + 2 μl of pol to start → kill with 25 μl cycle seq stop sol

same (enz) as p. 155 and 5 more 2 fold dilutions

Tube 15	Tube 14		units/rxn			
20 units/50λ rxn	2	1	.5	.25	.125	.063	.031

Taq = 1-15 .0156 .0078 .0038 0.00194 0.000

.000484 .000242 ← tube 1

Tne = 16-29

To Pag N

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	8/14/95	[signature] Recorded by	8/11/95

om: Hartman, Chris
o: Lasken, Roger
c: Rashtchian, Ayoub
ubject: Exo minus Klenow Kocton Dickinson Conditions
ate: Monday, August 07, 1995 3:40PM
i rity: High

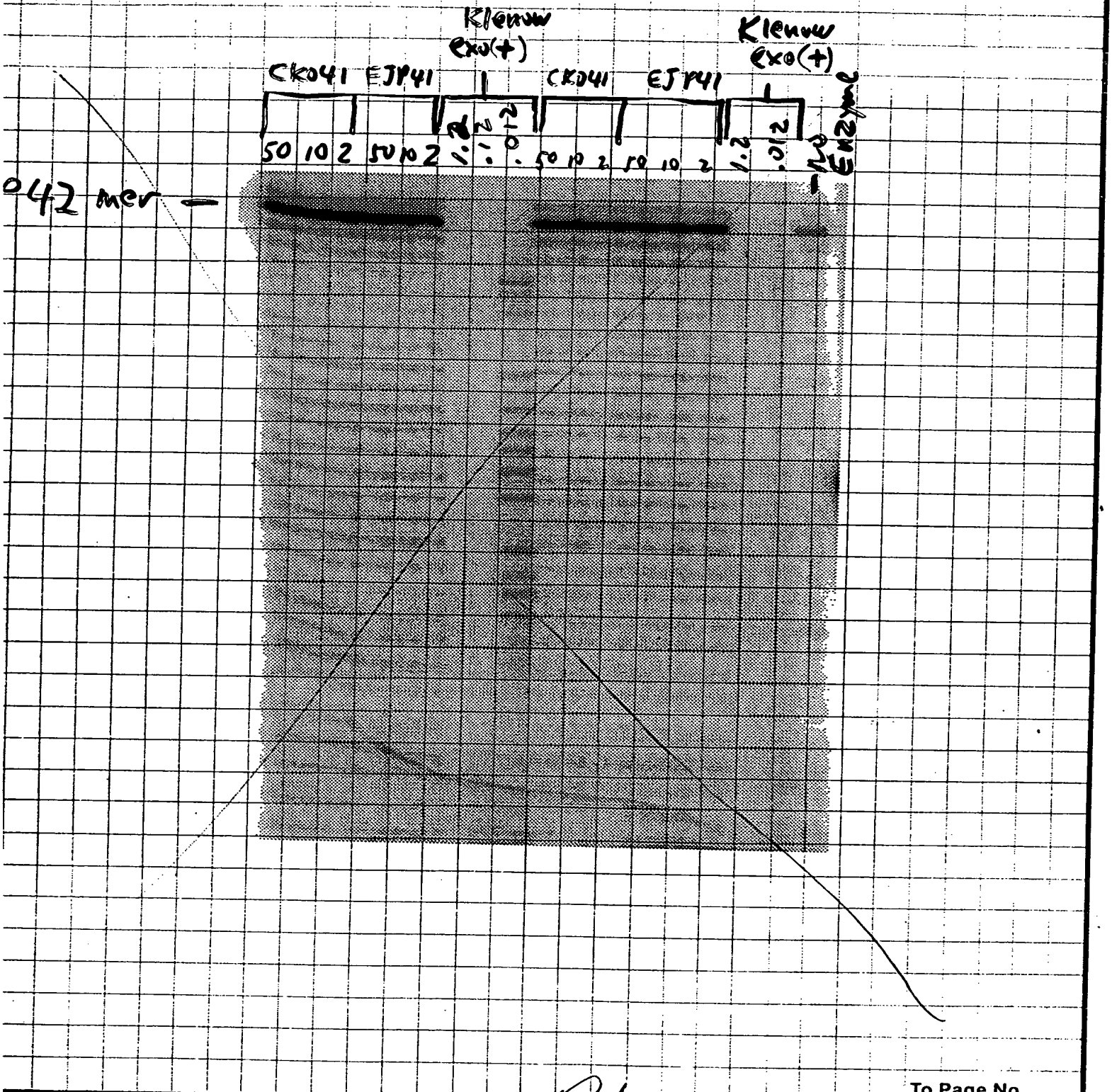
roject N .— Exhibit 156
Book N .— Appl. No. 09/558,421

P65,10
63

Taq PCR buffer

ger, The unit values for the bulk exo minus klenow are as follows:

Lot No.	U/ul
CK041	75
EJP41	130



To Page No.

& Understood by me,
Polanp

Date 8/14/95

Invented by

Recorded by

Date 8-14-95

Exhibit **157**
Appl. No. 09/558,421

Project
Book No.________ TITLE Test supermix for [dATP], [dGTP] [dCTP]

From Page No.__

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

7 ① (-dATP) 45 ⟶
② -dGTP 45 ⟶
③ (-dCTP)

4dNTPs — —
2.5 μM 5 5
5 μM 5 5
10 μM 5 5
20 μM 5 5
40 μM 5 5
60 μM 5 5

*Mix B #11 — —
2.5 5 5
5 5 5
10 5
20 5
40 5
60 5
H₂O 5 5 5 5

50 μl

1. assemble on ice
2. put in 9600 4°C
ramp to 70°C → 45''
ramp to 4°C

*for B
its 220 μM
(at 1.1x)
∴ for 110 μl
30 μl B
80 μl H₂O
Vf = 110 μl

To Page No.

Witnessed & Understood by me, J.D. Palamp
Date 8/21/95
Invented by [signature]
Recorded by
Date 8-15-95

43 is mix ① incl only if no 70°C before EDTA

44 is mix ③ incl only

45 2 hr # Rxn # 15 } w/o TCA

46 2 hr # Rxn # 29 }

To Page No.

d & Understood by me,	Date	Invented by	Date
Polaup	8/21/95	R corded by	12-11-95

Project No._____
Book No._____ TITLE Double P 65

From Page No.__

pmol

4 NTP	1	103.00	3.5
	2	264.00	14.8
	3	275.00	15.5
	4	638.00	41
	5	1025.00	68
	6	1248.00	84
	7	2048.00	140
B	8	56.00	0.2
	9	204.00	11
	10	297.00	17
	11	619.00	40
	12	1011.00	67
	13	1430.00	96
	14	1879.00	128
4 dNTP	15	94.00	2.9
	16	488.00	30
	17	653.00	42
	18	1332.00	89
	19	2200.00	150
	20	3902.00	273
	21	5706.00	395
B	22	83.00	2.1
	23	457.00	28
	24	643.00	41
	25	1289.00	86
	26	2218.00	151
	27	4406.00	304
	28	4082.00	282
BKGD	29	53.00	
2 λ	30	57231.00	42.9 cpm/pmol
4 dNTP	1	768.00	8.1
	2	854.00	32
	3	926.00	53
	4	928.00	53
	5	979.00	68
	6	1443.00	198
	7	1721.00	277
B	8	818.00	22
	9	763.00	6.8
	10	971.00	65
	11	931.00	54
	12	1155.00	117
	13	1230.00	138
	14	1473.00	207
	~~15~~	~~16.00~~	
BKGD	16	739.00	not very high ^3H dATP BKGD
2 λ	17	14195.00	

$$\frac{(14155\ \text{cpm})\ (50\ \mu l\ \text{Rxn} + 10\ \mu l\ \text{EDTA})}{(2\ \lambda\ \text{spotted})\ (40000\ \text{pmol ut/Rxn})} = 10.64\ ^3H\ \text{cpm}$$

To Pag

Witnessed & Und rsto d by m ,	Date	Invented by	Date
		R corded by	

g N .__

To Page No.____

ed & Und rst od by me,	Date	Invented by	Date
		Record d by	

166

Project No.
Book No.
TITLE Determination of how to Kill DNa

From Page No.

DNase I will be used to treat Tne enz prep. If DNA contamin is the cause (primer DNA) of the smear, than the DNase I treatment may eliminate the smear. 1st we'll establish how to kill DNase I, aft after it has been mixed w/ Tne, so it won't be active during R

1 DNase I rxn will be killed w/ CDTA + heat for 5, 10 + 30 mi
A second DNase I rxn will be killed ~~only~~ by heat only for 5, 10, 3
After the Killing treatment, the rxn will be mixed with ØX174 RF
If the DNase I was Killed, the ØX174 won't be degraded, even after a 3hr incubation.

materials: ØX174, 0.25 ug/ul in 0.1mM CDTA from LTI Lot FA370

DNase I, 1 u/ul in SB = 20mM NaOAc pH 6-5
5mM CaCl2
50% glycerol

25mM CDTA – 50ul 0.5M CDTA pH 8
950ul H_2O

200mM Tris 8.5 (note: the DNase I buffer is 8.4)
200ul 1M Tris 8.5
800ul H_2O

20mM $MgCl_2$ – 200ul 50mM $MgCl_2$
+ 300ul H_2

200mM KCl – ~~200mM~~ 66.7ul 3M KCl
+ 933.3ul H_2O

10x DNase I buffer = 200mM Tris-HCl pH 8.4
LTI 20mM $MgCl_2$
Lot EK2410 500mM KCl – it was a bit bubbly after mixing

0.8% TAE agarose gel w/ EtBr

To Page N

Witnessed & Understood by m , Date 8/21/95
Invented by Date
Recorded by 8/15/95

Page N

DNase I rxns: in 9600 PCR tubes

14 ul H_2O
+ 2 ul 10x DNase I buffer
+ 4 ul DNase I, 4 u/ul C_f = 0.2 u/ul
20 ul
Kill with heat + EDTA

(2) 14 ul H_2O
2 ul 10x DNase I buffer
4 ul DNase I C_f = 0.2 u/ul
20 ul - only heat kill this rxn

→ 15' at RT (= 23°C) → during this time 1 ug DNA should be digested

remove 4 ul (= 0.8 units DNase I)
+ 4 ul 0.25 ug/ul ØX174
+ 1.6 ul 10x DNase I buffer
+ 10.4 ul H_2O
20 ul w/ 1 ug

series to see how long it takes active DNase I to degrade 1 ug ØX174

→ immediately remove 4 ul + 1 ul 10x loading dye w/ 100 mM EDTA = 0 Kill time
0 time incubation w/ ØX174

→ 2' ~~later~~ remove 4 ul + 1 ul LD = 0' Kill time 2' w/ ØX174

→ 15' ~~later~~ remove 4 ul + 1 ul LD = 0' Kill time 15' w/ ØX174

→ 1 hr ~~later~~ remove 4 ul + 1 ul LD = 0' Kill time 1 hr w/ ØX174

→ 3 hr remove 4 ul + 1 ul LD = 0' Kill time 3 hr w/ ØX174.

(1) Kill the remaining 16 ul w/ 1.36 ul 25 mM EDTA C_f = 2 mM

(1)+(2) heat to 75°C in 9600

↳ 5' of heat kill, remove 4 ul for rxn (1)
+ 4 ul ØX174
+ 4 ul 200 mM KCl C_f = 50 mM, 16 ul
+ 1.6 ul 200 mM Tris 8.5 C_f = 20 mM, 16 ul
+ 2.02 ul 20 mM $MgCl_2$ C_f = 2.02 mM for 20 ul
+ 4.4 ul H_2O
20 ul → 3 hr at RT °C

To Page No.

sed & Understood by me,	Date	Invented by	Date
[signature]	8/21/95	Recorded by Carolyn Combs	8/15/95

From Page No.

I remove 4ul aliquots and treat in the same way after 15' and 30' killing with heat and EDTA

for rxn ②

For the DNaseI rxn that was killed by only heat, take 5', 15', and 30' killing time points by removing 4ul of rxn to a tube w/

4ul ΦX174, 0.25 ug/ul
4ul 200mM KCl
1.6ul 200mM Tris 8.5
1.6 ul 20mM $MgCl_2$
4.8 ul H_2O
20ul

- 3hr incubation at RT
- +3ul 10x Loading dye w/ 100 EDTA
- run 23ul on 0.8% gel

gel order

14 wells

1Kb ladder 10ug	1ug ΦX174	5' heat +EDTA	5' heat only	15' heat +EDTA	15' heat only	30' heat +EDTA	30' heat only	OK:11 0ΦX174	OK:11 2' w/ ΦX174	OK:11 15' w/ ΦX174	OK:11 1hr w/ ΦX174	OK:11 3hr w/ ΦX174	1Kb ladd

active DNaseI incubated w/ 1ug ΦX174 v varying amounts of time

To Pag N

Witn ss d & Understood by me,

Dat 8/21/95

Inv nt d by

R corded by

Dat 8/15/95

From Page No. _____

(note −20°C #11 assayed on p 52)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

−20 #1E 27/1 — 2 2 2
−20 #4E 27/1 — 2 2 2
−20 #7E 27/1 — 2 2 2

1.1X J-P-95 — 2 2 2
1.1X Fold Test — 2 2 2
("old" on P 34)

rTag 1/125 did — 2 2 2 2 2
J-30-95

Tag Reminder P120, 9 — 48 hrs →

			CPM	relative to Tag u/ml	P122, 9 (time 0) u/ml		
−20 1E	25	5911.00					
	26	6883.00	6531	.03	.037	81%	see P 53 where #11 of 27/1 54% and (reviewer #1E 4 show bette
	27	6801.00					
−20 4E	28	5982.00					
	29	5759.00	5649	.026	.029	90%	
	30	5205.00					
−20 7E	31	6079.00					
	32	5062.00	6187	.028	.033	85%	
	33	7422.00					
1.1X J-P-95	34	4974.00					
	35	4594.00	4773	$\frac{4773}{7686} .04 = .022$	$\frac{.022}{.023 (P54)}$	96%	
	36	4752.00					
1.1X old Field Test	37	4389.00					
	38	4552.00	4637	$\frac{4637}{8686} = .0213$	⇒	93%	
	39	4971.00					
rTag	40	8930.00					
	41	8601.00					
	42	8299.00	8686 ave	.04 (by definition)			
	43	8980.00					
	44	8616.00					
BKGD	45	78.00					
	46	100897.00					
2X mix	47	102480.00					
	48	102152.00					

To Pag

Witnessed & Understood by me,	Date	Invented by	Date
	8/21/95	Record d by	7-11-95

From Page No.___

specific activity of A mix = $\frac{101843 \text{ cpm}}{40{,}000} \left(\frac{50\mu l}{2\mu l}\right) = 63.7$ cpm/pmol nt

pmole = $\frac{\text{cpm}}{S.A} \left(\frac{60}{20}\right)$ (dilution)

SAM	CPM	
1	3072.00	147
2	1533.00	72
3	1127.00	53
4	516.00	24
5	198.00	9.32
6	3423.00	161
7	1581.00	74.5
8	1174.00	55
9	475.00	22.4
10	249.00	11.7
11	3178.00	150
12	2007.00	95
13	2979.00	140
14	2332.00	110
15	2799.00	132
16	2601.00	122
17	2954.00	139
18	2798.00	132
19	3532.00	166
20	1251.00	58.9
21	280.00	13.2
22	3472.00	164
23	2974.00	140
24	2605.00	123

8/17/95 cc

A no killing
A 90° 5'
A 90° 10'
A 90° 30'
A 90° 1hr
B no killing
B 90° 5'
B 90° 10'
B 90° 30'
B 90° 1hr
A 75° 30'
A 75° 1hr
A 75° 2hr
A 75° 4hr
B 75° 30'
B 75° 1hr
B 75° 2hr
B 75° 4hr
Tne, no heat, no EDTA
Tne, 90°, 10'
Tne 90° 1hr
Tne 75° 1hr
Tne 75° 4hr

140 pmole $\left(\frac{23.1}{2}\right)$ / 101,000 × 3 = 0.494 u/ul (expected 0.

A = 50 units Tne : 5 units DN
B = 50 units Tne : 1 units D

Nicking assay - after trea
to kill DNase I, the rxn
incubated w/ 1ug ΦX174
for 3hr at 23°C to ox
any remaining DNase I

ΦX174 nicked
ΦX174 supercoiled

note that no untreated
DNA was run as a positi
control for how 1ug looks
the Tne lanes serve as t
control because Liz Flynn show
Tne has no endonuclease
The 75°C 4hr treatment
EDTA and the lower leve
DNase was best for kil
the low level of DNase degrades 1ug easily

8/17/95 cc

To Pag

Witn ss d & Und rst od by m , Polanz

Date 8/21/95

Inv nt d by

Recorded by Pamela

Dat 8/17/95

Repeat assay of [illegible]
with -20°C, 4°C side by side

le N	Rxn#	
4°C	1-3	(2 pl assayed)
20°C	4-6	
4°C	7-9	
20°C	10-12	
4°C	13-15	
20°C	16-17	
4°C	19-21	
20°C	22-24	
ase	25	
ase	26	
ock	27	
nock	28	
Nase	29	
Nase	30	
ock	31	
nock	32	
stock	33	
stock	34	
ul stock	35	
stock	36	
stock	37	
stock	38	

		SAM	CPM1	ave	-20/4C
11E	4	1	7816.00	8440	90%
		2	9112.00		
		3	8393.00		
	-22-	4	7582.00	7558	
		5	7182.00		
		6	7910.00		
7E	4	7	6265.00	6317	102%
		8	5771.00		
		9	6916.00		
	-22-	10	6005.00	6443	
		11	6410.00		
		12	6913.00		
7E	4	13	7478.00	7711	96%
		14	7917.00		
		15	7738.00		
	-20-	16	7016.00	7385	
		17	7461.00		
		18	7679.00		
11E	4	19	5526.00	5211	98%
		20	5095.00		
		21	5012.00		
	-22-	22	5396.00	5100	
		23	5050.00		
		24	4855.00		
	30' Drain	25	2887.00	2835 ave	70% *
		26	2783.00		
	30' mock	27	2672.00	2814	
		28	2956.00		
	4 hr Drain	29	3095.00		
		30	3280.00		
	4 hr mock	31	2833.00		
		32	3113.00		
	36 u/ml	33	4234.00		
		34	3968.00		
		35	4606.00		
	5 u/ml	36	4839.00		
		37	3683.00	4041 x	
		38	4399.00		
		39	472.00		
		40	139229.00		

70% 5 u/ml stock dilution
2ul stock 5-7-
19.28ul Taq dil b
21.28ul
↳ 2ul
+45ul Taq dil b
47ul of 0.02 u/
ie 2835 ÷ 4041 = 70% recov
after "30' Drain" treatment
at 70°C

ons:
and 4hr Tne treated with
eI and mock rxns:
2ul enz, 0.47 u/ul P.175 NB11
45ul Taq dil buffer
47ul of 0.02 u/ul

1ul Tne stock was diluted
0.47 u/ul and then to
u/ul → 2ul 36 u/ul stock of Tne 5-7-95
+ 12.4ul Taq dil buffer
14.4ul of 5 u/ul
↳ 2ul of 5 u/ul + 19.28ul Taq dil buffer / 21.28ul of .47 u/ul, then diluted ↳ 2ul enz / 45ul Taq

To Page No._____

d & Understood by me,	Date	Invented by	Date
Polanyo	8/21/95	Recorded by	8-17-95

g N.__

atment of Tne with DNase I: 2 rxns of 87.6 ul H_2O
10 ul 10x DNase I buffer
1.39 ul Tne, 36 u/ul, 5-7-95 prep
1 ul DNase I, 1 u/ul
100 ul

test in PCR non-killed

remove 10ul to 4°C

C_f = 0.5 u/ul Tne
0.01 u/ul DNase I
2.5mm $MgCl_2$
1.2% glycerol
0.05mm $CaCl_2$

90ul → 1 rxn 30 15min RT
1 rxn 4hr RT (+15min)
↓
+ 5.74ul 50mM EDTA C_f = 3mM
new vol = 95.74ul $C_{f\,Tne}$ = 0.47 u/ul
↓
→ at 75°C for 4hr in 9600 PCR machine
12pm – 4pm, put on ice

trol reactions without DNase I: 2 rxns of 88.6 ul H_2O
10ul 10x DNase I buffer
1.39 ul Tne, 36 u/ul, 5-7-95
100 ul

mock reaction

remove 10ul from each to test in PCR
ie no killing treatment

90ul → 1 rxn 30 15min RT
1 rxn 4hr RT (+15min)
↓
+ 5.74 ul 50mM EDTA $C_{f\,Tne}$ = 0.47 u/ul
↓
at 75°C for 4hr in 9600
put on ice

To Page No.____

d & Understood by me,	Date	Invent d by	Date
Polanco	8/21/95	Recorded by Carolyn Conf	8/17/95

From Page No.__

PCR rxns: 0.5, 1, 2, 4 units Tne
done 8/17/95 long smear conditions and conditions to make specific
- test DNase I treated Tne
- test Tne that has been through a mock DNase I treatr
- test fresh Tne

mix A - to make a long smear
- for 14 rxns 1151.22ul H_2O
28ul 1M Tris 8.5 Cf = 20mM
23.38ul 3M KCl Cf = 50mM
29.4ul 50mM $MgCl_2$ Cf = 1.05mM
28ul 10mM dNTPs Cf = 200uM
1260ul

note: in the rxn with 4 units Tne, the [KCl] will be 55mM

mix B - to make 380bp product
- for 14 rxns 1043.98ul H_2O
28ul 1M Tris 8.5
39.62ul 3M KCl Cf = 85mM
30.4ul 50mM $MgCl_2$ Cf = 1.3mM
28ul 10mM dNTPs Cf = 200uM
28ul 20uM anchor primer Cf = 400n
28ul 20uM 6681 primer Cf = 400nM
28ul 50pg/ul mI3RF Cf = 100pg/rx

dilution of fresh Tne
36.4ul 5-7-95 stock
2ul stock
151.2ul Taq 513
153.2ul of 0.47u/ul

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
mix A	90																							
mix B													90											
-H_2O	8.9	7.87	5.74	1.49	8.9	7.87	5.74	1.49	8.9	7.87	5.74	1.49	8.9	7.87	5.47	1.49	8.9	7.87	5.47	1.49	8.9	7.87	5.47	1.49
DNase treated Tne	1.1	2.13	4.26	8.51									1.1	2.13	4.26	8.51								
mock treated Tne					1.1	2.13	4.26	8.51									1.1	2.13	4.26	8.51				
fresh Tne									1.1	2.13	4.26	8.51									1.1	2.13	4.26	8.51

100ul rxns started on ice.

To Pag A

Witness d & Understo d by m , Date 8/21/95 Invented by Dat

R cord d by Pamela Lamb 8/18/95

e N

in Lab is 9600 - 1 min 94°C

35 or 40 cycles (30 sec 94°C
30 sec 55°C annealing
2 min 72°C elongation
4°C

> program 76
method links 71, 75, 74

50 mM KCl
1.05 mM $MgCl_2$

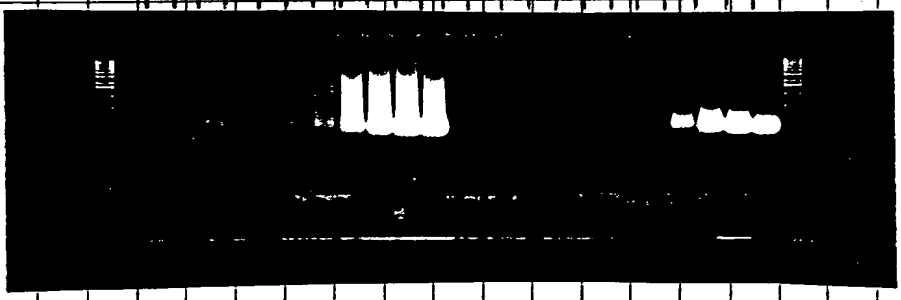
85 mM KCl
1.3 mM $MgCl_2$

Tne treated w/ DNase I for 30' 50u Tne 1 u DNase I

-95 enzyme units

Tne w/ DNase | mock | fresh | Tne w/ DNase | mock | fresh

0.5 1 2 4 0.5 1 2 4 0.5 1 2 4 0.5 1 2 4 0.5 1 2 4 0.5 1 2 4



8/18/95 cc

Mock rxn = Tne without DNase I taken through all the DNase I treatment steps

Fresh = untreated Tne used directly from -20°C stock

e: 85 mM KCl did not prevent the small smear from forming

To Page No.

d & Und rstood by me,	Date	Invented by	Date
Polcyn	8/21/95	Recorded by Caroline Combs	8/18/95

Exhibit 164
Appl. No. 09/558,421

Projec[t] 3'P 33 correct. mp19 for Klenow ex[o]

Book No.______ TITLE strand displacement assays

From Page No.__

follow P. 138, 9

	lot CK041	lot EJP41	no enzyme	
	①	②	③	4 x /
				= 2.1 pr
Rxmix (B&D system)	147.5 µl	147.5 µl	19.67 µl	0.2
* see P. 75				1750 / 7
Klenow exo(-) lot				
CK041 P.63, 75 u/µl dil in Taq SB to 4 u/µl	2.5			20
EJP41 P.63, 130 u/µl dil in Taq SB to 4 u/µl		2.5	0.33 µl Taq SB	
	150 µl	150 µl	20 µl	
			2.2 µl BJ	

41°C in 9600
remove 20 µl to 2.2 µl 10xBJ + 100 mM EDTA — start 1:2
at 1, 2, 5, 30, 90, 2 hr

	well# 1	2-7	8-13
load on 8% Agarose	③	①	②

To Page

Witnessed & Understood by me, [signature] Date 8/21/95
Invented by [signature] Date 8-21-95
Recorded by

xperiments on DNase I treated Tne
Mg^{2+} titration & mixing expt

N ___

pose: To determine if & how Tne was damaged by the DNase I
2/1/95 treatment. p. 178 mock rxns show low polymerase activity in the PCR, so the 75°C. treatment w/ EDTA affected Tne even if DNase I was not present. A unit assay showed most of the activity was still present (~25% died). Today, we'll add back Mg^{2+} in case there was more free EDTA than we thought. We'll also try poisoning a fresh Tne rxn w/ the treated Tne.

titration: 1.05, 1.3, 2mM MgOAc
smear & product conditions
1 unit DNase treated Tne (30' treatment)
1 unit fresh Tne from 5 u/ul stock

mix for 12 rxn → 24ul 1M Tricine pH 9 Cf = 20mM
30ul 2M KOAc Cf = 50mM
24ul 10mM dNTPs Cf = 200uM
882ul H_2O
960ul

B
80ul mix
4.7ul 25mM MgOAc Cf = 1.05mM
2.3ul H_2O
be 90ul/rxn

C
280ul mix
18.2ul 25mM MgOAc
16.8ul H_2O
Cf = 1.3mM

D
280ul mix
+ 28ul 25mM MgOAc
7ul H_2O
Cf = 2mM

mix B + 3 ul Tne treated w/ DNase 30' + 7ul H_2O = 100ul *
mix D + 3ul Fresh Tne Δ + 7 ul H_2O
mix C + 3ul treated Tne + 7ul H_2O
mix C + 3ul Fresh Tne + 7ul H_2O
mix D + 3ul treated Tne + 7ul H_2O
mix D + 3ul Fresh Tne + 7ul H_2O

* Tne at 0.33 u/ul, as determined by unit assay p. 73 NB 10 & p. 180 NB 11
Δ diluted 5 u/ul stock to 0.33 u/ul
5ul of 5 u/ul Tne 5-7-95
70.8ul of Taq SB
75.8ul of 0.33 u/ul Fresh Tne

To Page No. 180

& Und rstood by me, olmo | Date 8/21/95 | Invented by | Date 8/21/95 | Recorded by Emily Combs

From Page No.__

SAM	CPM1		
1	2952.00	2846	} Tne (DNase Treated 30') dil 1/23.5
2	2739.00		
3	4871.00	4115	} Liq Tne stock 5.4 u/l 5-7-95 diluted to 0.47 u/l (same as DNase treated above and then diluted 1/23.5
4	3459.00		
		68%	unit assay same as P73, 10

conclude Tne lost ~70% activity from killing 4hr killing of DNase I at 75°C (P175)

product mix for Mg^{2+} titration:

for 12, 100ul rxns = 24 ul 1M Tricine pH 9 Cf = 20mM
51 ul 2M KOAc Cf = 85mM
24 ul 10mM dNTPs Cf = 200uM
24 ul 20uM 6684 primer Cf = 400nM
24 ul 20uM anchor primer Cf = 400nM
24 ul M13RF, 50pg/ul Cf = 100pg/rxn
789 ul H_2O
960 ul

(E) for 3.5 rxns
280 ul mix
14.7 ul 25mM $MgCl_2$
20.3 ul H_2O Cf = 1.05mM
315 ul
use 90ul/rxn

(F)
280 ul mix
+ 18.2 ul 25mM $MgCl_2$
16.8 ul H_2O
315 ul Cf = 1.3mM

(G)
280 ul mix
+ 28 ul 25mM $MgCl_2$
+ 7 ul H_2O
315 ul Cf = 2mM

T Pag N

itn ss d & Understo d by m ,	Date	Invent d by	Dat
[signature]	8/28/95	R corded by [signature]	8/2/95

g No.

actions → DT = Tne treated w/ DNase for 30' p. 175, 1 unit, 0.33¼l
FT = Fresh untreated Tne diluted to 0.33¼l, 1 unit

smear condition = ~~50~~ 85mm KOAc, 20mm Tricine pH 9.0, no template, no primers

product condition = 85mm KOAc, 20mm Tricine pH 9, anchor primer + 6681 primer on m13

OAc] mM	1.05		1.3		2 mM		1.05		1.3		2	
me	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT
	1 unit of enz/rxn											
) ✗	1	3	4	6	7	9	10	12	13	15	16	18

20ul of each 100ul rxn was run on a 0.8% gel p. 184 →

We had also planned to do rxns with 4 units of the DNaseI treated Tne, but there was not enough of the enz. to set up these rxns

Mg2+ was omitted from rxn by mistake

xng experiment to determine if the DNaseI-treated Tne has a "poisenous" substance in it – mix untreated Tne w/ DNase-treated Tne. smear & product conditions 1 u untreated + 0 treated
.5 "
1 "
2 "
4 "

nix for smear, 6 rxns = 12ul 1M Tricine pH 9 Cf = 20mM
1.5ul 2M KOAc Cf = 50mM
12ul 10mM dNTPs Cf = 200uM
441ul H_2O
480ul → use 80ul/100ul rxn

7
5 80ul mix + 3ul fresh Tne (0.33¼l) + 17ul H_2O
80ul mix + 3ul " + 1.52ul treated Tne + 15.48ul H_2O ← see top of page
80ul mix + 3ul " + 3ul " + 14ul H_2O
80ul mix + 3ul " + 6ul " + 11ul H_2O
80ul mix + 3ul " + 12ul + 5ul H_2O

T Page No.

ed & Understood by me,	Date	Inv nt d by	Date
Polanyi	8/28/95	Recorded by Carolyn Combs	8/21/95

From Page No. 181

2nd try at the mixing expt. - I forgot to add MgOAc to the rxns on

smear buffer: Cf = 20mM Tricine pH 9.0
50 mM KOAc
1.05mM MgOAc
200uM dNTPs

→ no primers or template
mix 1 unit of fresh Tne w/
0, 0.5, 1, 2, 4 units of
mock-treated Tne. p. 17.

for 6 rxns = 12ul 1M Tricine pH 9 ✓
15 ul 2M KOAc ✓
25.2ul 25mM MgOAc ✓
12ul 10mM dNTPs ✓
415.8ul H_2O ✓
480ul → use 80ul mix per 1, 100ul rxn

product buffer: Cf = 20mM Tricine pH 9
85mM KOAc
1.3mM MgOAc
200uM dNTPs
400nM 6681 primer
400nM anchor primer
100 pg/rxn M13mp19 RF template

→ mix 1 unit fresh Tne w/
0, 0.5, 1, 2, 4 units
of mock treated Tne
p. 1

A unit assay was done on the mock treated Tne on Fri 8/18/95 p. 75 N
We'll assume a concentration of 0.33 u/ul, which was the concentration on 8/18. The Wase treated Tne had not lost any more acti
at 4°C over 3 days, as shown by unit assay on 8/21/95 p. 180. It's likely
the mock didn't loose an

for 6 rxns = 12ul 1M Tricine pH 9 ✓
25.5ul 2M KOAc ✓
31.2ul 25mM MgOAc ✓
12ul 10mM dNTPs ✓
12ul 20uM 6681 ✓
12ul 20uM anchor ✓
12ul 50 pg/ul M13 RF in TE ✓
363.3ul H_2O ✓
480ul, use 80ul mix / 100 ul rxn

To Pag N

Witnessed & Understood by m
Dat 8/28/95
Inv nted by
Dat 8/22/95
R cord d by

g No.

	19	20	21	22	23	24	25	26	27	28	~~29~~
mix ✓	80ul —				1						
ct mix ✓						80ul —				1	
Tne 4u/ul ✓	3ul (1unit) —									1	
K-treated .33u/ul	0	1:52	3	6	12	0	1:52	3	6	12	
✓	17	15.48	14	11	5	17	15.48	14	11	5	
	100 ul										

94°C 1 min
94°C 30 sec
55°C 30 sec } 35 cycles
72°C 2 min
4°C - hold

~~thod 76, Lab 15 9600~~ Method 103 Lab 16 9600

lution of fresh Tne: 5ul of 5u/ul Tne 5-7-95 Lig stock
70.8ul Taq SB
75.8 ul of 0.33u/ul fresh Tne

5% TAE gel w/ EtBr, 20ul of each rxn was run on gel

To Page No.

ed & Understood by me,	Date	Invent d by	Date
	8/28/95	Recorded by Carolyn Combs	8/22/95

Project No. _____

84 Book No. _____ TITLE _____

From Page No. _____

Results:

Mg^{2+} titration p. 179

DT = Tne treated 30' with DNase I p. 175

FT = fresh, untreated Tne

	smear cond: 50mM KOAc inc template/primers				product cond 85mM KOAc			
[MgOAc] mM	1.05		1.3	2	1.05		1.3	2
1 unit enz →	DT	FT	DT	FT	DT	FT	DT	FT

Mixing expt. p. 182

1 unit Fresh Tne + increasing units of mock treated Tne (no DNase)

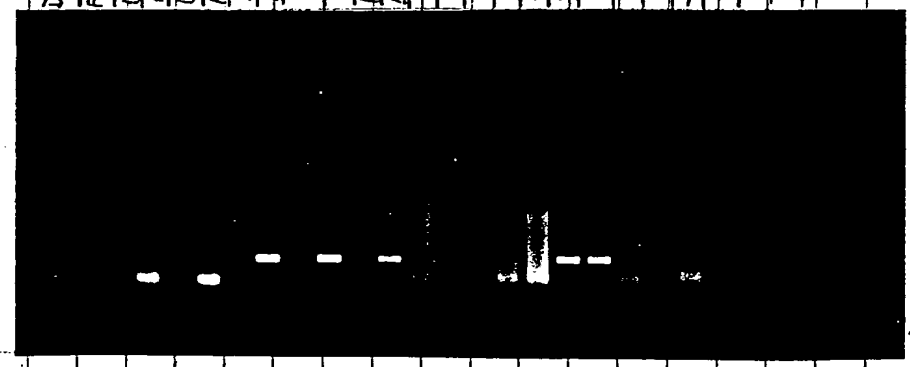
smear cond units of mock treated					product cond units of mock treated				
0	.5	1	2	4	0	.5	1	2	4

RNase treatment of Tne p. 185

+ = Tne treated w/ RNase

− = Tne not treated w/ RNase

smear		product	
+	−	+	−



CSE 8/23/95

Conclusions from Mg^{2+} titration expt:

A unit assay was done on the DNase I treated Tne (see p 182) and fresh Tne. Equal units of the DNase I treated and fresh Tne were used in the Mg^{2+} titration PCR reactions. The treated Tne did not make any specific product, while the Fresh Tne did. We conclude that the 75°C incubation with CDTA (to kill the DNase I) damages Tne. Therefore we do not yet know if treating Tne w/ DNase can eliminate the "bad seed" DNA and prevent a smear.

If there was some residual DNase I activity, the product & smear could be degraded. 2 pieces of data argue against the active DNase explanation. 1) our nicking assay p. 174 shows that 1ug of ΦX174 was not degraded in 3hr and was only nicked a little (~10%) at the DNase I killing treatment

2) in a previous expt (p. 177) the mock-treated Tne showed some low activity in a PCR as the DNase I treated Tne. So, the DNase was not responsible for the low amount of smear products made.

Tne + CDTA died at 94°C, maybe adding Mg^{2+} back can overcome the damage to Tne p. 177

Further expts to try: → measure thermostability of the 75°C w/ CDTA treated Tne, by cycling the Tne to 94°C before doing the unit assay

→ purify the DNase I-treated Tne away from DNase or "poisen"-gel filtration

To Page No.

Witnessed & Understood by me,	Dat	Invented by	Dat
	8/28/95	Recorded by Pamela	8/23/95

Expt.: RNase A + 1 ± treatment of Tne. Is RNA the bad seed that primes the smear.

Project No._____

PCR 1 is 1+2

PCR 2 is 3+4

PCR 3 is 5+6

PCR 4 is 7+8

See result on p.184

of 3 → RNase

#1, ~~2~~

50ul worth of buffer w/ 100ul worth of dNTP, target, primers

smear) V V 1ul Tricine

V V 1.25ul 2m KOAc

V V 2.1 ul 25mM MgCAc

V V 2 ul 10mM dNTP

V V 2ul 6681 → 20uM

V V 2ul anchor

V V 2 ul m13, 50ng/ul

1 RNaseT1 } both diluted 10 fold in 10mM Tricine

1 RNase A }

35.65 ul H_2O

50ul

T1 1460u/ul

A 10mg/mL = 10ug/ul

1 ul → 40ug RNA/mL

dil 10x use 1ul

#2

or Tne V V 1 ul Tricine

V V 1.25 ul 2m KOAc

V V 2.1 ul 25mM MgOAc

Enzyme → ~~1~~ 2 ul Tne Liz 5u/l → want 1 unit → dil to 0.5u/l in SB Tne.

1 ul RNase I

1 ul RNase A } dil 10x in 10mM Tricine

ul H_2O

V V 41.65 ul

50

15' 37°C → ice → mix → PCR

same w/o RNase

#3, 4

ESC 8/22/95

d & Und rstood by me,	Dat 8/28/95	Invented by	Date
Polanyo		Recorded by Linda Emmons	8/22/95

From Page No.__

8/22/95 CS

(#5)

expt 3 RNase:

for product

1 ul Tricine 1M
✓✓ 2.13 ul 2M KOAc
✓✓ 2.6 ul 25mM MgOAc
✓✓ 2 ul dNTP 10mM
✓✓ 2 ul 6681 20uM
✓✓ 2 ul anchn 20uM
✓✓ 2 ul m13 50 pg/ul
1 RNase
1 RNase
✓✓ 34.27 H_2O
50 ul

✓✓ 1 ul Tricine
✓✓ 2.13 2M KOAc (#6)
✓✓ 2.6 25mM MgOAc

2 ul Tne 0.5 U/l

✓✓mix 40.27 H_2O
1 RNase
1

✓same w/o RNase (#7), (#8)

To Pag

Witnessed & Understood by me, Dat 8/28/95 Invented by Dat

Recorded by Laurie Paul 8/22/95

e N 184

nclusions from the mixing expt. on p. 184:

The purpose of the mixing expt was to see if 0.5, 1, 2, 4 units of mock reated Tne could poison a PCR with 1 unit of fresh Tne. The mock treated Tne received the 4hr 75°C CDTA treatment but did not contain any DNase I.

The mock-treated Tne did not poison the ability of fresh Tne to make a $\wedge^{10.3}$ smear under the standard smear buffer conditions of 50mM KOAc, 20mM Tricine, 1.65 mM MgOAc. The differences in smear intensity are probably just representative of typical variation in smear intensity. Therefore the mock-treated Tne does not contain a poison that is effective at the levels tested.

The mock-treated Tne also did not poisen fresh Tne's ability to make a specific product (.5u mock w/ 1u fresh still made product. The smears seen with 1, 2, 4u mock probably ~~are the~~ result from having too many total units. Using more than 1 unit Tne/100ul rxn always results in a smear. ~~The unit of mock was not enough to make a~~ smear. 1 unit mock may not be exactly the same as 1 unit of treated (which doesn't show any PCR activity in the Mg^{2+} titration expt) and the window for ~~tear~~ activity is probably very narrow. — More controls + expt should be done to confirm the absence of a poisen.

nclusions from RNase expt — Tne treated with RNase A + RNase T1 and then used directly in a PCR. If RNA is the "bad seed" RNase might cure the formation of a smear and increase product yield. The RNase treatment had no effect on either smear formation or product yield. We conclude that RNA is not priming the smear reaction

To Page No.

d & Understood by me,	Date	Invented by	Date
Polanyi	8/28/95		8/22/95
		Recorded by Evelyn Ennis	

Exhibit **167**
Appl. No. 09/558,421

Project No.________

Book No.________ **TITLE** ________

From Page No.__

Results:

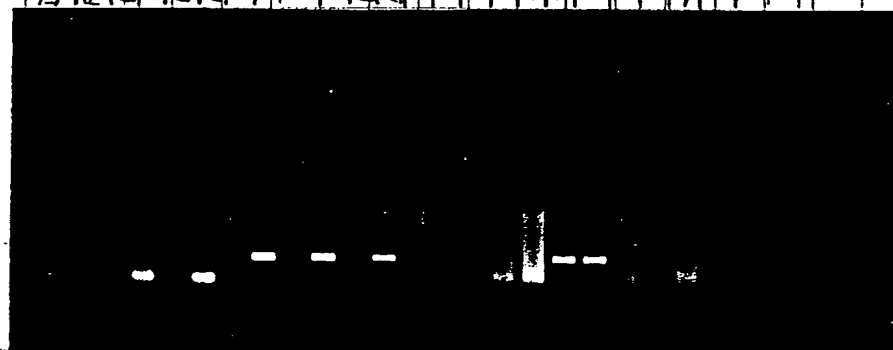
Mg^{2+} titration p. 179
DT = Tne treated 30' with DNase I p.175
FT = fresh, untreated Tne
smear cond. 50mM KOAc no template/primer | product cond 85mM KOAc

Mixing expt. p. 182
1 unit Fresh Tne + increasing units of mock treated Tne (no DNase)
smear cond | product cond
units of mock treated

RNase treatment of Tne p. 185
+ = Tne treated w/ RNase
− = Tne not treated w/ RNase
smear | product

[MgOAc] mM: 1.05 | 1.3 | 2 | 1.05 | 1.3 | 2

1 unit enz → DT FT DT FT DT FT DT FT DT FT DT FT | 0 .5 1 2 4 | 0 .5 1 2 4 | + − | + −



CSC 8/23/95

Conclusions from Mg^{2+} titration expt:

A unit assay was done on the DNase I treated Tne (see p. 182) and fresh Tne. Equal units of the DNase I treated and fresh Tne were used in the Mg^{2+} titration PCR reactions. The treated Tne did not make any specific product, while the Fresh Tne did. We conclude that the 75°C incubation with CDTA (to kill the DNase I) damaged Tne. Therefore we do not yet know if treating Tne w/ DNase can eliminate the "bad seed" DNA and prevent a smear.

If there was some residual DNase I activity, the product & smear could be degraded. 2 pieces of data argue against the active DNase explanation. 1) our nicking assay p. 174 shows that 1µg of ΦX174 was not degraded in 3hr and was only nicked a little (~10%) after the DNase I killing treatment

2) in a previous expt (p. 177) the mock-treated Tne showed the same low activity in a PCR as the DNase I treated Tne. So, the DNase was not responsible for the low amount of smear products made.

Tne + CDTA died at 94°C, maybe adding Mg back will overcome the damage to Tne p. 174

Further expts to try: → measure thermostability of the 75°C w/ CDTA treated Tne, by cycling the Tne to 94°C before doing the unit assay
→ purify the DNase I-treated Tne away from DNase or "poison" - gel filtration

To Page No.

Witnessed & Understood by me, [signature] **Date** 8/28/95

Invented by

Recorded by [signature] **Date** 8/23/95

ig N. __

2.2 X reactions, V_f = 0.1 each

Rxmix (B&D)

Cf at 1X

-2 HPO4 pH7.6 P.61	~~33 μl~~	16.5 μl ✓		50 mM
dNTPs 10mM	~~13.2 μl~~	6.6 ✓		200 μM each
BSA nuclease free acetylated Stratagene cat 300041-57	~~4.4 μl~~	2.2 ✓		0.1 mg/ml
50% glycerol	~~170.84 μl~~	~~90.42~~ 86.9 ✓		13.17 % ← (includes contributed by Klenow ex.)
1 M MgCl2 (Mg goes in after PO4 is diluted to avoid ppt of MgPO4)	~~4.60~~	2.31 ✓		7 mM
33 annealed mp19 3:9: 4λ/50μl of Rxn vol 1.12 pmol primer 1.24 pmol circle ratio primer = 2	~~52.8 μl~~	26.4 ✓		
H2O	~~340.14~~	183.6 ~~180.1~~ ✓		
V_f =	~~649~~	324.5		

note 5 μl Kl / 295 μl Rxn contributes (5/300) 50% glycerol = 0.833% to Cf
∴ overall Cf is 14% glycerol in Rxn

To Page No._____

sed & Understood by me,	Date	Invented by	Date
[signature]	8/28/95	[signature] Recorded by	7-28-95

Exhibit 169
Appl. No. 09/558,421

Project No.______
Book No.______

TITLE Repeat ^{32}P dNTP incorp of truncated in Tne of P7778 with Δt

From Page No.__

mix A — for 4 Rxns (Cf 100λ

10 X PCR buffer	✓	40
50 mM MgCl$_2$	✓	56
H_2O	✓	260
		356

(Cf = 1100 CPM/pmol 4
Cf 200 μM each

use 95 μl/100 μl Rxn

	①	②	③		
A	89 μl	⟶		✓	(93.6 u)
Tne Ciz 36 u/λ	2.6			✓	
Tne PL7-22-15 19 u/λ (see P 127, 11)		5		✓	95 u
rTaq EKBT1 dil to 36 u/λ			2.6	✓	93.6
Taq SB	2.4		2.4	✓	heat reaction 1' 94 then lower heat to 7 then start reaction
dCTP, dNTP mix*	6	⟶			by addition of ^{32}P dCTP, dNTP mix
	Vf 100 μl				

* mix
^{32}P dCTP 10 mCi/ml — 16
10 mM dNTPs — 8
Vf = 24

remove 10 μl to 5 μl cycle seq stop solution at 1 2 5 15 30 60 90 min

run of 8% PAGE

with ddA, ddT and PFII (Reactions #22, 23, 2 at top of P 7P) reloaded here as number 22, 23, 24 also.

is no Enz

To Page N

Witnessed & Understood by me, [signature] Date 8/28/9?

Invented by [signature] Date 8-28-9?

Recorded by

Exhibit 170
Appl. No. 09/558,421

86

see P155-157 w/ Ultima and P164 for Tne Taq

Project No.

Book No.

TITLE Processivity Tne FY vs Ultima

From Page No. 600K11 from P.75

Mix A process (for 37 Rxns, 40 μl/50 μl R...

^{32}P 33 mer correct • mp19 ✓ ✓ 130 μl — 7.8 pmol circles ; 3.9 pmol prime...
circle / primer = 2
.06 pmol circle A

H_2O	✓ ✓	1109.5 μl	for 37 Rxns
10 x PCR buffer	✓ ✓	148 (1x at 40 μl V)	use 40 μl / Rxn 50.1
50 mM $MgCl_2$	✓ ✓	55.5 μl (1x at 50 μl V$_f$)	C_f = 1.5 mM Mg
10 mM dNTPs	✓ ✓	37 (1x at 50 μl)	1x at 50 μl Rxn = 2...

V_f = 1.48 ml

		3.6 U/s Tne Liz well #	Tne FY 9.4 U/s	Ultima 5 u (x)
	.00005 U/μl	#1	12	23
. pol dil	.0001	2	13	24
(dil in	.0002	3	14	25
Taq SB	.0004	4	15	26
	.0008	5	16	27
	.0016	6	17	28
	.0032	7	18	29
	.0064	8	19	30
	.0128	9	20	31
	.0256	10	21	32
	.0512	11	22	33

for 40 μl mix ...
= 0.21 pmol
50 μl ...
~ same as ...

1.25
put 2 μl pol into 8 μl of x PCR buffer
preheat to 70°C 1 min in 9600

start with 40 μl of Mix A process (also preincubated to 70
stop at 2 min with 25 μl cycle seq stop sol.

number "0" is Mix A process 40 μl
Taq SB 2
H_2O 8
50 μl
cycle seq stop 25 μl

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	9/7/94	[signature] Record d by	8-28-95

Exhibit 171
Appl. No. 09/558,421

38

Project N
Book No.______ TITLE Label Fidel pri for incorp opposite Template dU

From Page No.__

Fidel Pri (P54) 100 μM (its a 27mer) — 5 μl ✓ (500 pmol total)

γ-32P ATP 10 mCi/ml (3.33 μM) — 10 ✓ (33 pmol total)

5X Kinase buffer — 10 ✓
PNK 10u/λ — 1
H2O — 24 ✓

50 μl — 37°C, 30' → 70°C, 5'

	dT	dU	
	25	25	✓
Fidel Temp (dT)	5		
Fidel Temp dU		5	

500 (~~1000~~ pmol) (Temp/pri = 2)

P54 100 μM
10 mM tris pH8 247.78 → μl ✓
vf 277.8 → μl

Cf = 900 nM primer

↓
90°C 2 min
↓
cool slow

use 5 μl / 50 μl extension reaction for Cf = 90 nM primer

To Page No

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	9/7/95	[signature]	8-31-95
		Recorded by	

Project No.
Book No. TITLE

From Page No.

(14 Rxns) Chem I — 14 II 14 Rxns

Same as p. 20 except it has dNTPs at 1mM each at 5x

5 x Chem (+dNTPs)	70 µl		✓	1.0 mM dNTPs	
10 x PCR buffer		70	✓		
50 mM MgCl2		14.7	✓	1.0 mM	
10 mM dNTPs		14	✓		
Human DNA (spleen) 20 ng/µl	7 14	7 14	✓		
10 µM w/3267bp 16/12	14 28	14 28	✓		
H_2O	534 532 28	552.3 545.3 503.5 28	✓	400 nM primer	
	672	672			

use 47 µl/Rxn 5 µl

Chem I # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

I 48 →

II 48 → 45

534 Temp SB

Tne wt (Lig 1-7-95)

0.25 U/µl	2	6	6	2			0.4
0.5	2	6	6	2			2.5 µl of 5 U/µl Tc
1	2	2.5	10	2			
1.5	2	2	4.67	2			
2	2	2	3	2			

Tne Q5 FY 5.4 U/µl SB

0.25	5.57	5.5	2	2			
0.5	5.5	5.5	2	2			
1	1.2	10.0	2	2			
1.5	1	5.27	2	2			
2	1.5	5.55	2	2			
3	2	4.27	2	2			
4	2.5	3.37	2	2			
	50 µl						

To Page No

Witnessed & Understood by me,	Date	Invented by	Date
Rob Olano	9/7/95	Rec rd d by	5-4-95

PCR with OAPUH, glycerol, Chen vs TAC buffer
for Tne wt and ΔSFY
see P 78, 11 for wt conditions

Proj ct N. _____
B ok No. _____
93

ag No. _____

94°C 1 min

94°C 30 s
55°C 30 s } 35 cycles
72°C 2 min

4°C hold

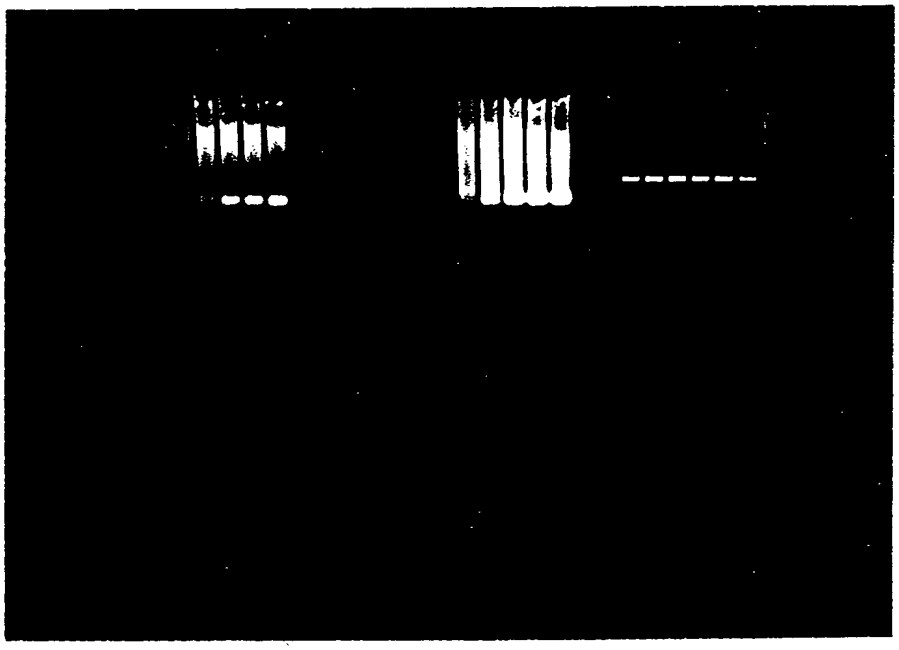
(get BJ+EDTA added ≤ 30' after finish in case 3' bkgr was probl

Chen buffer | Taq PCR buffer

Tne: wt ΔSFY wt ΔSFY

units

2 units Taq pol in Taq PCR buffe



← 267

Result: only get expected 267 bp product for Tne ΔFY if PCR buffer used consistent with lower ionic strength of PCR buffer helping the less processive deleted pol (Chen is 85 mM KOAc compared to 50 mM

To Page No. _____

sed & Understood by m ,	Date	Invented by	Date
Polanco	9/7/95	Recorded by	9-14-95

Exhibit 173
Appl. No. 09/558,421

Project PCR with Tne ∆5 FY

04 Book No.______ TITLE 267bp – 7.5 bp products

From Page No.__

	10 min	~~20~~		
Mix A	10x PCR buffer	100	✓	for 20 PCR
	~~5x Cheng + dNTP~~	~~200 μl~~	✓	
	50 mM MgCl2	21 μl	✓	(1.05 mM)
	Human DNA 80 ng/λ	20 μl	✓ ✓	
	H2O	810.5 ~~251.5~~	✓ ✓	
9.4u/λ	Tne ∆5 FY	8.5 μl	✓ ✓	
		980 μl		

	1	2	3	4	5	6	7	8	9
mix A	49 μl →								
267 bp 16/12 h/g	1								
BDNF 10 μM primers		1							
1.366 Kb primers			1						
2.0				1					
2.82					1				
4.1						1			
5.5							1		
6.166								1	
7.5									1

start 8:40
for 7 min elong need ~ 5 hrs
done ~ 2:30

94°C, 1'

94°C 30S
55°C 30S } 35 cycles
72°C 2 min (#1-9) or 7 min (#10-18)
start 11:35 (start 11:25)

need ~ 2½ hr for 2 min e
(done ~ 11:10)

4°C (get BJ + EDTA in ≤ 30' after finish in case 3' exo is a problem

T Pag No

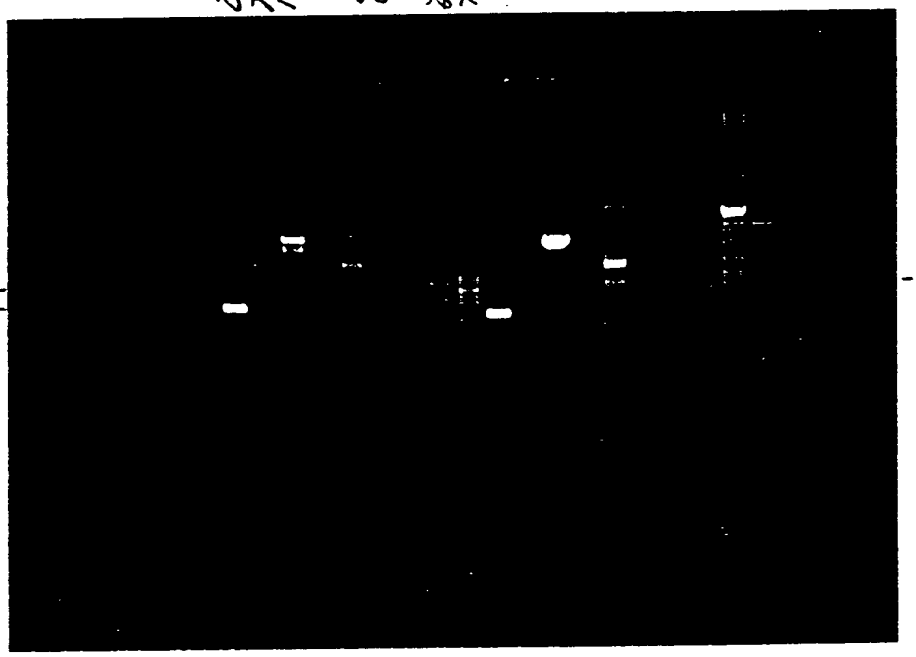
itnessed & Understood by me, [signature] | Dat 9/7/95 | Invented by [signature] | R cord d by | Dat 9-6-95

No.

2' elongation

7' elongation

267
800
1.36
2
2.8
4.1
5.5
6.1b
7.5



267	+	
800	+	(for 7 min elong
1,36	+	
2	+	(for 7 min
2.8	—	
4.1	—	
5.5	—	
6.1b	—	
7.5	—	

To Page No.

ed & Understood by me,

Dat 9/7/95

Invented by

Recorded by

Date 9-8-95

From Page No.__

10 x Taq PCR buffer = 200 mM Tris 8.4, 500 mM KCl

10 x ultima buffer is 100 mM Tris pH 8.8, 10 mM KCl

∴ I will try 0 10 25 50 mM KCl cf
(for now I'll stay with 20 mM cf Tris 8.8

	[A]	
1 M Tris pH 8.8	20 µl ✓	(cf = 20 mM) (for 10 x 1
50 mM $MgCl_2$	21 µl ✓	(cf = 1.05 mM)
4 dNTPs	25 ✓	(250 µM ea)
Human DNA 80 ng/µl	5 ~~10~~	
9.4 u/µl Taq DSFY	8.5 µl ✓	(4 u/50 µl as per P
H_2O	86.5 µl ✓	∴ 8 u/100 µl PCR
	540	use 94 µl/100 µl P

	①	②	③	④	⑤	⑥	⑦	⑧
[A]	94 µl →							
KCl	—			—			✓	
200 mM		✓			✓		✓	cf = 1
500 mM			✓			✓	✓	2
1 M				✓		✓	✓	✓
primers								
2.67 kb 16/12 h/g	1 →							
1.36 kb				1 →				
H_2O	✓			✓				
	100 µl							

cycle as per P 94 with 2 min elongation

start 7:40 AM done

To Pag

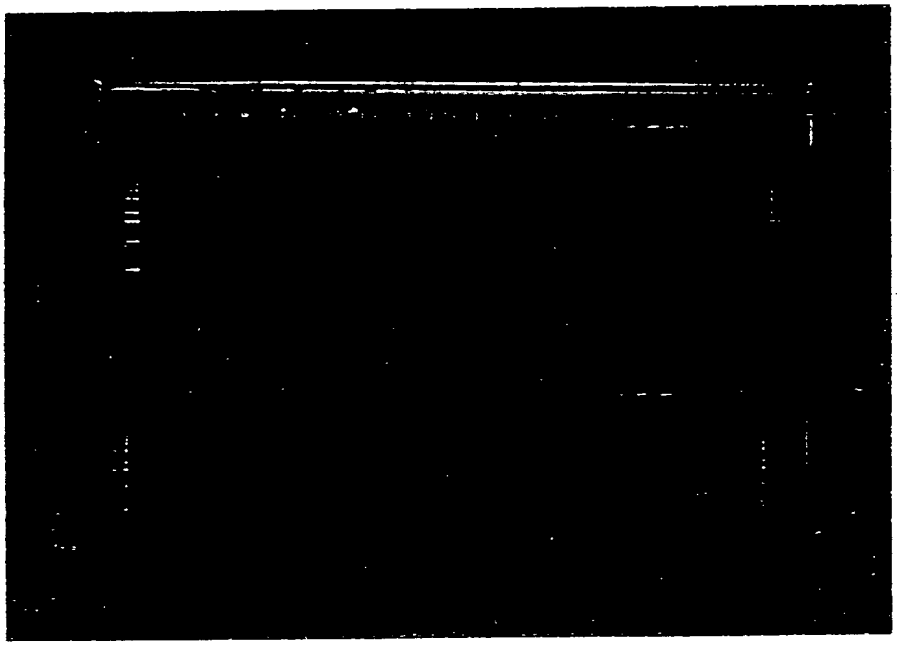
Witnessed & Understood by me,	Date	Invented by	Date
[signature]	9/7/95	Recorded by	9-7-95

No.

Result

note 267 bp product does just begin to appear at 5 cycles at highest Ne Cl level of 50 mM

concluded:
1) higher NaCl helps in contrast to expectation that it would inhibit this destructive form of Taq.
2) target DNA is limiting here since high yields were obtained for 4X more DNA in P93 and 94



← 267

apparently used ≥1 µl of H/S DNA (70ng)/ 50µl Rxn
used only 0.25µl Taq

To Page No.

d & Understood by me,	Date	Invented by	Date
[signature]	9/7/95	Recorded by	9-7-95

From Page No.__

They want 10,000 units and same SB as LTI except include 100 mM KCl

Therefore

T th (formerly thought to be Tf1) 2.5 ml (10825 units total
4-30-95 (see P8 for units)
4.33 u/µl

*. 2 M KCl in 50% glycerol and 20 mM Tris pH 7 0.132 ml

V_f = 2.632 ml

final units = 4.1 units/µl

* KCl 0.298 g ✓ (MW = 74.55)
glycerol 1 ml ✓
H_2O to 2 ml ✓
1M Tris pH 7 20 µl ✓

V_f = 2 ml

To Page N

Witnessed & Understood by me,	Date	Invented by	Date
Deborah Polaski	9/9/95	Recorded by	9-9-95